

# Large Loss of Dissolved Organic Nitrogen from Nitrogen-Saturated Forests in Subtropical China

Yunting Fang,<sup>1,3\*</sup> Weixing Zhu,<sup>2</sup> Per Gundersen,<sup>3</sup> Jiangming Mo,<sup>1</sup>  
Guoyi Zhou,<sup>1</sup> and Muneoki Yoh<sup>4</sup>

<sup>1</sup>South China Botanical Garden, The Chinese Academy of Sciences, Guangzhou 510650, China; <sup>2</sup>Department of Biological Sciences, State University of New York—Binghamton, Binghamton, New York 13902, USA; <sup>3</sup>Forest & Landscape Denmark, Faculty of Life Sciences, University of Copenhagen, Hørsholm Kongevej 11, 2970 Hørsholm, Denmark; <sup>4</sup>Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan

## ABSTRACT

Dissolved organic nitrogen (DON) has recently been recognized as an important component of terrestrial N cycling, especially under N-limited conditions; however, the effect of increased atmospheric N deposition on DON production and loss from forest soils remains controversial. Here we report DON and dissolved organic carbon (DOC) losses from forest soils receiving very high long-term ambient atmospheric N deposition with or without additional experimental N inputs, to investigate DON biogeochemistry under N-saturated conditions. We studied an old-growth forest, a young pine forest, and a young mixed pine/broadleaf forest in subtropical southern China. All three forests have previously been shown to have high nitrate ( $\text{NO}_3^-$ ) leaching losses, with the highest loss found in the old-growth forest. We hypothesized that DON leaching loss would be forest specific and that the strongest response to experimental N input would be in the N-saturated

old-growth forest. Our results showed that under ambient deposition ( $35\text{--}50\text{ kg N ha}^{-1}\text{ y}^{-1}$  as throughfall input), DON leaching below the major rooting zone in all three forests was high ( $6.5\text{--}16.9\text{ kg N ha}^{-1}\text{ y}^{-1}$ ). DON leaching increased 35–162% following 2.5 years of experimental input of  $50\text{--}150\text{ kg N ha}^{-1}\text{ y}^{-1}$ . The fertilizer-driven increase of DON leaching comprised 4–17% of the added N. A concurrent increase in DOC loss was observed only in the pine forest, even though DOC:DON ratios declined in all three forests. Our data showed that DON accounted for 23–38% of total dissolved N in leaching, highlighting that DON could be a significant pathway of N loss from forests moving toward N saturation. The most pronounced N treatment effect on DON fluxes was not found in the old-growth forest that had the highest DON loss under ambient conditions. DON leaching was highly correlated with  $\text{NO}_3^-$  leaching in all three forests. We hypothesize that abiotic incorporation of excess  $\text{NO}_3^-$  (through chemically reactive  $\text{NO}_2^-$ ) into soil organic matter and the consequent production of N-enriched dissolved organic matter is a major mechanism for the consistent and large DON loss in the N-saturated subtropical forests of southern China.

**Key words:** nitrogen deposition; nitrogen additions; nitrogen saturation; DOC and DON; subtropical forest; southern China.

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\*Corresponding author; e-mail: fangyt@scbg.ac.cn

## INTRODUCTION

Nitrogen (N) biogeochemical research has traditionally been centered on the dynamics of inorganic N (mostly  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ), with soil N mineralization and nitrification, plant uptake of inorganic N, and  $\text{NO}_3^-$  losses as the key processes studied in terrestrial ecosystems (Vitousek and Reiners 1975; Aber and others 1998; van Breemen 2002). Human activities have dramatically altered the global N cycles through fertilizer application, expanded biological N fixation, and industrial combustion processes (Vitousek and others 1997; Gruber and Galloway 2008). In heavily human-occupied areas, increased atmospheric N deposition into natural terrestrial ecosystems has led to higher nitrification rates and higher  $\text{NO}_3^-$  leaching loss, reinforcing the inorganic N-centered conceptual model of N cycling (Aber and others 1998; Fenn and others 1998; Gundersen and others 1998).

Studies of N cycling in unpolluted areas have challenged the inorganic N-centered model (Hedin and others 1995; Perakis and Hedin 2002; van Breemen 2002). Based on the stream chemistry data from 100 unpolluted primary forests in Chile, South America, Perakis and Hedin (2002) demonstrated that DON export was the predominant pathway of N loss in these forests, in contrast to  $\text{NO}_3^-$  dominance in stream export in Northern Hemisphere temperate forests. The persistent DON loss, even under extremely N-limited conditions, has been proposed as an important mechanism leading to broad N limitation in temperate forests (Vitousek and others 1998; Perakis and Hedin 2002). In other parts of the world, Lovett and others (2000) found both spatial and seasonal variations of  $\text{NO}_3^-$  concentrations among 39 streams in the Catskill Mountains of New York, but consistent concentrations of DON. Pellerin and others (2006) reported that N loading explained little variability in surface water DON concentration in 206 forested-watersheds across various geographical zones in North America, and that DON accounted for the majority of total dissolved N (TDN) loss. In addition, the direct use of labile DON monomers such as amino acids by plants and microorganisms under N-limited conditions has challenged the traditional concept of N availability and N cycling (Schimel and Bennett 2004).

Several recent studies in N-polluted areas demonstrated that DON could be important for N transportation and as a critical component of the N budget under N-saturated conditions; N fertilization experiments showed a consistent and large increase in DON efflux following the increase of N

input (McDowell and others 2004; Pregitzer and others 2004). A recent analysis by Brookshire and others (2007) showed that elevated atmospheric N deposition (spanning an input gradient of 5–45 kg N ha<sup>-1</sup> y<sup>-1</sup>) increased DON output from temperate forested watersheds in the Appalachian Mts. of the USA. In contrast, no significant relation between throughfall inorganic N input and forest floor DON leachate export was found across 12 European sites (Park and Matzner 2006). Plot-scale studies on the effect of N fertilization on DON dynamics also yielded conflicting results: no change at one set of European sites (Gundersen and others 1998; Raastad and Mulder 1999; Sjöberg and others 2003), a decline at another site (Vestgarden and others 2001), and large and persistent increases at two North American sites as mentioned above (McDowell and others 2004; Pregitzer and others 2004). These widely divergent results of DON response to elevated N inputs suggest that there are unknown factors controlling the processes that drive the production and loss of DON in and from forest ecosystems.

We contend that DON production and loss in response to elevated inorganic N input depends on the initial ecosystem status, similar to nitrification and  $\text{NO}_3^-$  loss predicted under the N-saturation model (Aber and others 1998) but likely with different mechanisms. Under N-limited conditions, DON losses from watersheds are largely bio-unavailable compounds leaching from slow-turnover soil organic matter (SOM) in strict stoichiometric proportion with dissolved organic carbon (DOC, Hedin and others 1995; Rastetter and others 2005). Dominance of hydrologic N loss by DON is also expected if organic matter decomposition is slow, resulting in C losses as DOC rather than  $\text{CO}_2$ , with DON accompanying the DOC loss (Brookshire and others 2007). Therefore, DON losses are under the control of SOM dissolution/depolymerization and transport rather than the control of biological N demand (Brookshire and others 2007). At the remote sites in the Southern Hemisphere, DON loss from temperate forests is generally 0.2–3.5 kg N ha<sup>-1</sup> y<sup>-1</sup> (Perakis and Hedin 2002), which is similar to the background level of atmospheric N input (Vitousek and others 1997). Elevated atmospheric N deposition and experimental N input can alleviate N-limitation, but strong inorganic N immobilization by plants and microbes as well as DOM adsorption to mineral soils (Qualls and Haines 1992) might delay the leaching responses in the loss of both dissolved inorganic N (DIN) and DON.

Alternatively, several mechanisms operating under N-saturated conditions could result in significant DON loss. First, microbial turnover could increase under N saturation leading to increased DOC loss; there have been some reports of concomitant increase in DOC loss in N fertilized forests (Pregitzer and others 2004), although not in others (McDowell and others 2004). If DOC loss does increase under elevated N input (Findlay 2005), then DON export would be elevated following a simple stoichiometric balance (Brookshire and others 2007). Secondly, enhanced N mineralization could lead to more DON production in the critical depolymerization step before ammonification (Schimel and Bennett 2004). Some of this low molecular weight DON could end up in leachate. Thirdly, abiotic incorporation of excessive inorganic N, especially  $\text{NO}_3^-$  to SOM, may lead to the leaching loss of N-enriched dissolved organic matter (DOM). Abiotic immobilization has long been considered responsible for efficient retention of both atmospherically deposited and experimentally derived N (Aber and others 1998). There has been strong laboratory evidence that after  $\text{NO}_3^-$  addition, part of the  $\text{NO}_3^-$  was recovered in extractable soil organic N, indicating conversion of inorganic N into organic N (Berntson and Aber 2000; Dail and others 2001; Davidson and others 2003). Finally, diminished DON adsorption to mineral soils under long-term inorganic N deposition and increased atmospheric DON deposition (Neff and others 2002) can contribute to increased DON loss from forest soils.

Here we report DON and DOC leaching from forest soils exposed to long-term high ambient N deposition with or without additional experimental N input, to examine DON biogeochemistry under N-saturated conditions. This study was conducted as part of an ongoing N-addition experiment in three subtropical forests (a mature old-growth, a young mixed forest, and a young pine forest) in southern China (Fang and others 2006). To our knowledge, this is the first report on DON biogeochemistry in warm and humid forests under both chronically high-N deposition and experimental N additions. Most wet and humid tropical and subtropical forests differ from temperate forests with generally greater N supply and deficiency of other nutrients (Martinelli and others 1999; Matson and others 1999), higher nitrification rates (Vitousek and Sanford 1986), and larger hydrologic efflux. In China, high human population density and rapid economic development in the past quarter century have caused high atmospheric N deposition (up to  $50 \text{ kg N ha}^{-1} \text{ y}^{-1}$  measured as throughfall input)

(Huang and others 1994; Fang and others 2008). Thus the study at our sites gives us an opportunity to examine DON biogeochemistry under extremely N-polluted conditions. Our previous study showed that a considerable amount of  $\text{NO}_3^-$  leaching occurred in all three forests, being highest in the old-growth forest and under high-N treatment (Fang and others 2006). According to the mechanisms listed above, we hypothesize that (1) DON loss in our study sites would be forest-type dependent, with higher DON loss in mature old-growth forest than in younger mixed and pine forests; and (2) in all three forests, DON production and loss would respond quickly to experimental N additions, but with a more pronounced increase in the mature old-growth forest. DOM produced in forest soils can leach below the rooting zone and move into the aquatic ecosystem. The changes in the quantity and quality of DOM produced can then influence water quality, aquatic biodiversity, and ecosystem productivity (see Pregitzer and others 2004).

## METHODS

### Site Description

The study was part of a N addition experiment carried out in the Dinghushan Biosphere Reserve (DHSBR) in the Guangdong province, South China ( $112^\circ 33' \text{ E}$  and  $23^\circ 10' \text{ N}$ ). Three adjacent forest types (one old-growth forest and two young forests, 2–4 km apart) were chosen according to their initial N-saturation conditions (Fang and others 2006). The climate is warm and humid. The mean annual rainfall of 1,927 mm has a distinct seasonal pattern, with 75% falling from March to August and only 6% from December to February (Huang and Fan 1982). Mean annual relative humidity is 80% and mean annual temperature is  $21.0^\circ\text{C}$ , with average January and July temperatures  $12.6^\circ\text{C}$  and  $28.0^\circ\text{C}$ , respectively.

The old-growth forest is a regional climax type and has been protected for more than 400 years by monks in the nearby temples (Wang and others 1982). In this forest the major species are *Castanopsis chinensis*, *Machilus chinensis*, *Schima superba*, *Cryptocarya chinensis*, *Syzygium rehderianum* in the canopy and sub-canopy layers. Both young forests originated from the 1930s clear-cut and subsequent pine plantation (Wang and others 1982; Fang and others 2006). The colonization from natural dispersal of regional broadleaf species has changed plant composition in the mixed forest (dominated by *Pinus massoniana*, *Schima superba*, and *Castanopsis*

*chinensis*), whereas the pine forest is still dominated by *Pinus massoniana* under continuous human disturbances that lasted until 1990 (generally the harvesting of understory and litter, Mo and others 2003). The old-growth forest had a basal area of almost twice ( $26.2 \text{ m}^2 \text{ ha}^{-1}$ ) those in the pine and mixed forests ( $14.0$  and  $13.8 \text{ m}^2 \text{ ha}^{-1}$ ), but less litter accumulation in forest floor ( $8.9$ ,  $23$ , and  $20 \text{ Mg ha}^{-1}$  in the old-growth, pine, and mixed forests, respectively; Fang and others 2006).

The three forests have the same soil type (Oxisols), similar elevation (100–250 m above sea level), similar slope aspect (south-facing), and degree ( $18$ – $25^\circ$ ). The soil is lateritic red earth formed from sandstone (He and others 1982). The soil depths vary among the three forests, as well as within each forest. In the old-growth forest the soil depth ranges from 30 to 70 cm. The soil is about 40 cm deep in the mixed forest, and generally less than 40 cm in the pine forest. The soil is strongly acidic, with pH being lower in the old-growth forest (3.8) than in the pine and mixed forests (4.0, Fang and others 2008). In the upper 10 cm of mineral soil, the old-growth forest had significantly higher concentrations of total organic C, N, and P, but lower C/N ratio and bulk density than the two younger forests (Fang and others 2008). These soil variables did not differ significantly between the pine and mixed forests (Fang and others 2008).

These forests have been experiencing high ambient N deposition ( $21$ – $50 \text{ kg N ha}^{-1} \text{ y}^{-1}$  in throughfall, including organic component) for the past 15 years (Fang and others 2008). In an earlier paper, we reported DIN leaching of 16.5, 14.2, and  $50.9 \text{ kg N ha}^{-1} \text{ y}^{-1}$  in the pine, mixed, and mature forests, respectively (Fang and others 2006). This result combined with the soil N conditions described above suggested that the mature old-growth forest was in a more advanced N saturation stage than the younger forests.

### Experimental N Treatment

In all three forests, dissolved  $\text{NH}_4\text{NO}_3$  at levels of 50 (low-N) and 100 (medium-N)  $\text{kg N ha}^{-1} \text{ y}^{-1}$  was sprayed monthly on the forest floor beginning in July 2003. An additional high-N treatment ( $150 \text{ kg N ha}^{-1} \text{ y}^{-1}$ ) was applied in the old-growth forest to create an extremely N-saturated condition. In each forest, three replicate plots were randomly selected for each level of N treatment and three additional plots without experimental N input were set as controls. There were a total of 9 plots in the pine forest, 9 plots in the mixed forest, and 12 plots in the old-growth forests. Each plot measured

$10 \times 20 \text{ m}$  and was surrounded by a 10 m wide buffer strip. The overall experimental area in each forest was approximately 0.7 ha. During monthly N application, fertilizer was weighed, mixed with 20 l of water, and applied to the plots using a backpack sprayer below the canopy. Two passes were made across each plot to ensure even distribution. The control plots received the same amount of water without additional N (Fang and others 2006).

### DON and DOC in Soil Leachates

Soil leachate was collected at 20 cm depth, below the major rooting zones. Previous studies in these forests showed that more than 72% of the fine root biomass ( $<5 \text{ mm}$ ) was distributed in the 0–20 cm soil (Liao and others 1993; Wen and others 1999). Except for one medium N plot in the old growth forest where soil was too shallow, in each plot, two zero tension tray lysimeters ( $755 \text{ cm}^2$  per tray) were installed in April/May 2003, and concentrations and fluxes of DIN, DON, and DOC in soil leachate were quantified in the year 2005, 16 months after the first N application. Leachate was sampled twice a month, once prior to the N spray and the other 15 days after the spray. Leachate from the two lysimeters in the same plot was combined on the date of collection. Leachate was not available in the first two months and last three months of the study year due to dry weather conditions. We noted that by this method N export from these forests might not have been completely characterized; processes below this sample depth (for example, root uptake at deeper soil layer, sorption, desorption, and microbial decomposition) may further influence water chemistry and element export from the ecosystem (Hedin and others 1998). However, using a ceramic suction cup method to collect soil solution at 40 cm depth in 2004 in the pine and old-growth forests (the collection was terminated due to technical difficulties), we found a minor difference in annual volume-weighted DIN concentrations between the two soil depths (Fang and others 2008).

Samples were filtered within 24–48 h of collection through  $0.45 \mu\text{m}$  filters in the laboratory and frozen prior to later chemical analyses. The concentration of  $\text{NH}_4^+$  was analyzed using the indophenol blue method followed by colorimetry, and  $\text{NO}_3^-$  was analyzed after cadmium reduction to  $\text{NO}_2^-$ , followed by sulfanilamide-NAD reaction (Liu and others 1996). Total dissolved N (TDN) was determined using persulfate oxidation to  $\text{NO}_3^-$  followed by colorimetric determination (Liu and others 1996). Method detection limits were 0.01,

0.02, and 0.05 mg N l<sup>-1</sup> for NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and TDN, respectively. In the old-growth forest, NH<sub>4</sub><sup>+</sup> concentrations in a few samples were below the detection limit, in which case values were assigned to be 0.005 mg N l<sup>-1</sup> (half the detection limit). DON was calculated as the differences between TDN and DIN (NH<sub>4</sub> + NO<sub>3</sub> - N). DOC concentration was measured using high-temperature (680°C) catalytic (Pt) oxidation with a Shimadzu TOC-V instrument.

## Calculations and Statistics

Concentrations of soil leachate were multiplied by the recorded water volume for each sample collection, and then summed to determine annual N or C fluxes in kg ha<sup>-1</sup> for each plot. Because DON concentration was calculated as the difference between TDN and DIN, and TDN and DIN were measured by different methods, it led to some slightly negative values of DON (accounting for about 7% out of total 406 samples). In those cases, the zero values were assigned when we carried out flux calculations and repeated measurement ANOVA (RMANOVA), but were not included in the DON versus NO<sub>3</sub><sup>-</sup> and DON versus DOC regression analyses, nor in the calculation of DOC:DON ratios.

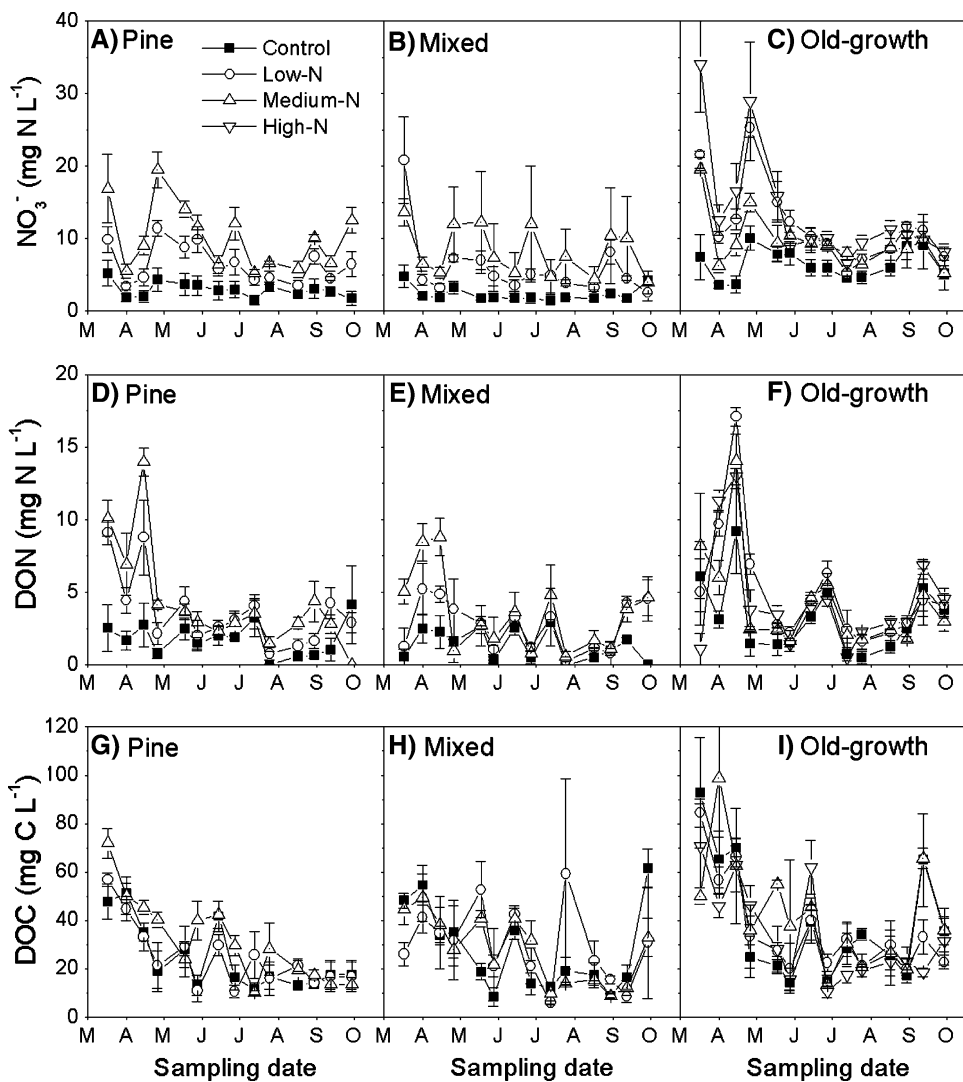
One-way RMANOVA with Tukey's HSD was used to test the effects of N treatment (fixed effect, independent variable) on the chemistry of soil leachate (dependent variable), in each of the three forests. One-way ANOVA with Tukey's HSD was used to test the effect of N addition on annual N and C effluxes. For DOC:DON ratios, we performed a two-way ANOVA using season and N treatment as main factors. We assigned March to May as spring, June to August as summer, September to November as autumn, and December to February as winter. We used linear regression analysis to examine the relationships between DON, DIN, and DOC variables. Residual plots were examined to check equal variance assumptions for ANOVA and to identify statistical data outliers. Outliers were removed from all statistical analyses but included in the figures and tables. All N treatment effects were separately analyzed within each of the three forests, where experimental plots were randomly assigned. Because forest types (old-growth, mixed, and pine forests) cannot be "truly replicated" in this study, we limited our comparisons among forests to control plots (those without experimental N additions) only. All analyses were conducted using SPSS 10.0 for Windows. Statistically significant differences were identified when *P* was less than 0.05 unless otherwise stated.

## RESULTS

### Concentrations in Soil Leachates

Ammonium concentrations in soil leachate (annual average ranged from 0.3 to 1.0 mg N l<sup>-1</sup>) were much lower than the NO<sub>3</sub><sup>-</sup> concentrations and were not presented here. Concentrations of both NO<sub>3</sub><sup>-</sup> and DON displayed a distinct seasonal pattern, with generally low concentrations in summer (June to August), and high concentrations in spring (March to May, Figure 1A–F). Mean NO<sub>3</sub><sup>-</sup> concentrations across the treatments and forest types were 9.7, 6.2, and 6.4 mg N l<sup>-1</sup> in spring, summer, and autumn, respectively, and mean DON concentrations were 5.0, 2.5, and 3.9 mg N l<sup>-1</sup>. In the control plots, RMANOVA across 14 sampling dates showed that old-growth forest had significantly (*P* < 0.001) higher DON concentrations than the younger forests, with the yearly average concentration in the old-growth forest (3.2 mg N l<sup>-1</sup>) being 1.8 and 2.3 times those in the pine (1.8 mg N l<sup>-1</sup>) and mixed forests (1.4 mg N l<sup>-1</sup>). Correspondingly, the average NO<sub>3</sub><sup>-</sup> concentration in the old-growth controls (6.5 mg N l<sup>-1</sup>) was 2.2 and 2.8 times those in the pine (2.9 mg N l<sup>-1</sup>) and mixed (2.3 mg N l<sup>-1</sup>) forests; the difference was significant (*P* = 0.028) between the old-growth and two younger forests but not between the two younger forests.

Experimental N additions caused an increase in DON concentration in all three forests (Figure 1D–F). In the pine and mixed forests, average DON concentrations in the low-N and medium-N treatments were 99–104% and 146–157% higher than in their respective controls, and the differences between medium-N treatment and control in these two forests were both statistically significant (*P* = 0.03 and 0.05 for the pine and mixed forests, respectively). In the old-growth forest, DON concentrations were highly variable across sample dates, treatments, and replicates (Figure 1F). In this forest, RMANOVA showed a significant effect of N additions only in the low-N treatment (*P* = 0.02). Average DON concentrations in the low-N, medium-N, and high-N treatments were 60, 35, and 39% higher than those in the controls. In comparison, concentrations of NO<sub>3</sub><sup>-</sup> increased by 49–254% in the N-addition plots of three forests compared to their respective controls (Figure 1A–C). The effect of N additions on NO<sub>3</sub><sup>-</sup> concentrations was significant in the pine and old-growth forests (except in the old-growth medium-N treatment, *P* = 0.002 and 0.012 for the pine and old-growth forests, respectively), but it was not significant in the mixed forest (*P* = 0.215).



**Figure 1.** Concentrations of  $\text{NO}_3^-$  (A–C), DON (D–F), and DOC (G–I) in soil leachate collected below the major rooting zone from the pine, mixed, and old-growth forests in 2005. Measurement began 16 months after the first N applications. Soil leachate data were available from March to September 2005. Means  $\pm$  standard errors,  $n = 3$  except in the medium-N treatment of the old-growth forest where  $n = 2$  (see text).

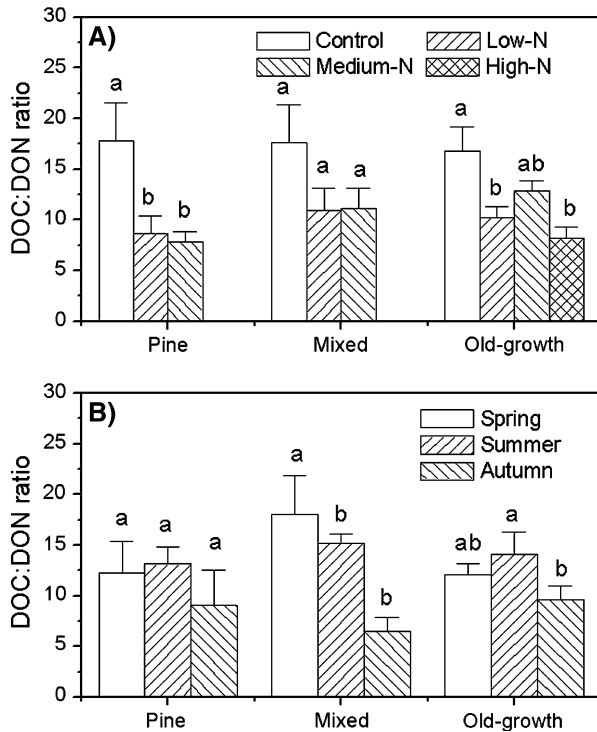
The DOC concentration in soil leachate displayed a similar seasonal trend as DON concentration (Figure 1G–I). In the control plots, the DOC concentration was significantly higher ( $P = 0.017$ ) in the old-growth forest (averaged  $39.1 \text{ mg C l}^{-1}$ ) than in the pine and mixed forests ( $24.5$  and  $27.4 \text{ mg C l}^{-1}$ , respectively). A significant increase (30%) in the DOC concentration after N additions was found in the medium-N plot of the pine forest ( $P = 0.013$ , Figure 1G). The addition of N had no significant effect in either the mixed or old-growth forest (Figure 1H, I). DOC:DON ratios declined following N additions in all three forests (Figure 2A); yearly average DOC:DON ratios were 16–18 in the control plots and 8–13 in N-addition plots. Two-way ANOVA (season and N treatment as main factors), analyzed separately for each forest, indicated significant N-treatment effects in the pine and old-growth forests ( $P = 0.031$  and  $0.001$ , respectively), and were marginally significant in

the mixed forest ( $P = 0.092$ , Figure 2). DOC:DON ratios were lowest in autumn (9, 6, and 10 in the pine, mixed, and old-growth forests, respectively), and highest in summer in both pine (13) and old-growth forests (14), and highest in spring in the mixed forests (18, Figure 2B). In the control plots, there was no significant difference in DOC:DON ratios among the three forests (Figure 2A).

DON concentrations were significantly ( $P$  from  $0.004$  to  $<0.0001$ ) correlated to the concentrations of  $\text{NO}_3^-$  and DOC both in the control plots and in the N-treatment plots across all three forests (data not shown). However, the regression coefficients were low ( $R^2$  from  $0.05$  to  $0.20$ ).

### Fluxes of N and C in Soil Leachates

DON fluxes in soil leachate varied depending on forest type and experimental N-additions (Table 1). In the control plots, DON flux in the old-growth



**Figure 2.** Effects of N treatment (A) and season (B) on the DOC:DON ratios of the soil leachate, analyzed separately for each forest. Different letters indicate significant difference ( $P < 0.05$ ). Error bar represents one standard error,  $n = 3$  except in the medium-N treatment of the old-growth forest where  $n = 2$  (see text).

forest was 2.0 and 2.6 times those in the pine and mixed forests (Table 1). Two-way ANOVA (season and N treatment as main factors), analyzed separately for each forest, showed that experimental additions of inorganic N significantly increased DON fluxes with no treatment  $\times$  season interactions ( $P = 0.009$ ,  $0.038$ , and  $0.043$  for the pine, mixed, and old-growth forests, respectively), although one-way ANOVA on DON fluxes over the entire year showed no significant difference (Table 1). DON fluxes were 102–162%, 59–61%, and 35–69% higher in the N-addition plots than in those corresponding control plots of the pine, mixed, and old-growth forests (Table 1). This fertilizer-driven increase was  $4.0$ – $13.6$  kg N ha<sup>-1</sup> y<sup>-1</sup>, which made up 4–17% of the experimental N input. This DON response to experimental inorganic N input was less pronounced than the NO<sub>3</sub><sup>-</sup> response, which ranged from  $13.2$  to  $53.5$  kg N ha<sup>-1</sup> y<sup>-1</sup> and comprised 18–53% of the experimental N input (Table 1).

The regression analysis showed that DON leaching increased significantly (in the pine forest,  $P = 0.05$ ) or marginally (in the mixed and old-growth forests,  $P = 0.095$  and  $0.061$ ) with elevated

DIN input (Figure 3A). Similar to DON loss, NO<sub>3</sub><sup>-</sup> leaching was also increased with DIN input (Figure 3B). DON leaching was highly correlated with NO<sub>3</sub><sup>-</sup> leaching, both when analyzed across all study plots ( $r^2 = 0.82$ ,  $P < 0.001$ ,  $n = 29$ ) or analyzed separately for each individual forest ( $r^2 = 0.77$ ,  $0.84$ , and  $0.82$ ;  $P = 0.001$ ,  $0.011$ , and  $< 0.001$ , in the pine, mixed, and old-growth forests, respectively, Figure 3C). These relationships were much stronger than those between DON leaching and DIN input (Figure 3A), suggesting that other factors aside from DIN input controlled both DON and NO<sub>3</sub><sup>-</sup> leaching. The slopes of these regressions were also forest-type dependent and the steepest slope was found in the pine forest.

DON leaching accounted for 23–38% of TDN flux, with the DON fraction slightly lower with increased N inputs (Table 1). In the control plots, especially in younger forests, the soil was a sink for throughfall DON (Fang and other 2008), with DON leaching fluxes accounting for 46, 45, and 84% of the throughfall input in the pine, mixed, and old-growth forests, respectively (Table 1). Additional N input (as inorganic N) diminished such DON retention, that is, by increasing DON leaching loss. In some N-addition plots, soil became a net source for DON leaching (Table 1).

In contrast to the DON fluxes, the effect of N addition on DOC leaching was not significant in any of the three forests in spite of a substantial increase in the concentration observed in the medium-N pine plots (Table 1). Nevertheless, DOC leaching flux was still correlated with DON flux in the pine forest ( $P = 0.008$ , Figure 3D), or when data from all three forests were combined ( $P < 0.001$ ).

## DISCUSSION

### The Response of DON Loss to Increased N Input

The present study demonstrated substantial DON loss from three subtropical forests in a warm and humid region of southern China exposed to long-term ambient atmospheric N deposition, and a consistent positive response to experimental N additions in these forest ecosystems. We contend that the response magnitude of DON production and loss to increased N inputs depend largely on the initial ecosystem N status and ecosystem type, similar to the increases of inorganic N production (N mineralization and nitrification) and NO<sub>3</sub><sup>-</sup> loss, but likely by different mechanisms (see discussion below). Thus, higher DON loss would be expected

**Table 1.** Entire Year (2005) Fluxes of Throughfall Input and Leachate Loss of DIN and DON (kg N ha<sup>-1</sup> y<sup>-1</sup>), DOC (kg C ha<sup>-1</sup> y<sup>-1</sup>), and DON/TDN Proportion in the Pine, Mixed, and Old-Growth Forests

Forest/N treatment	Input (throughfall + addition)			Leachate loss			
	DIN	DON	DON/TDN	NO <sub>3</sub> <sup>-</sup>	DON	DON/TDN	DOC
<i>Pine forest</i>							
Control	27	18.2	0.40	15.5(6.3)	8.4(3.4)	0.33(0.00)	111(13)
Low-N	77	18.2	0.19	36.2(8.1)	16.9(6.1)	0.29(0.03)	114(32)
Medium-N	127	18.2	0.13	61.1(0.3)	21.6(2.8)	0.25(0.02)	175(13)
<i>Mixed forest</i>							
Control	23	14.6	0.39	9.5(2.8)	6.5(2.0)	0.38(0.03)	95(29)
Low-N	73	14.6	0.17	22.7(2.0)	10.4(0.6)	0.29(0.01)	101(18)
Medium-N	123	14.6	0.11	32.6(14.9)	10.5(1.4)	0.27(0.06)	101(18)
<i>Old-growth forest</i>							
Control	32	20.1	0.39	41.7(7.1)	16.9(3.9)	0.28(0.02)	198(44)
Low-N	82	20.1	0.20	68.4(9.7)	23.6(2.5)	0.25(0.01)	164(16)
Medium-N	132	20.1	0.13	59.8(10.0)	22.8(5.5)	0.27(0.02)	256(43)
High-N	182	20.1	0.10	95.3(21.2)	28.5(4.7)	0.23(0.01)	185(15)

Standard errors in parentheses,  $n = 3$  except the medium-N treatment in the old-growth forest where  $n = 2$  (see text).

Throughfall data were from Fang and others (2008).

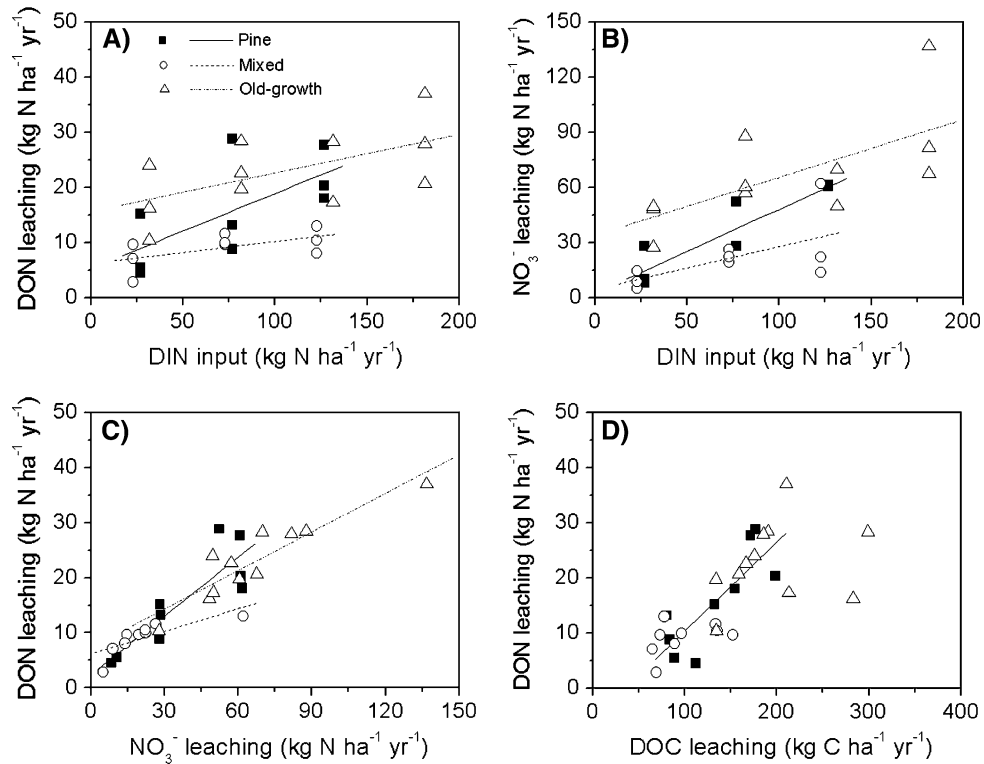
One-way ANOVA, using entire year flux data and analyzed separately for each forest, showed that N-treatment effect was significant only for leachate loss of NO<sub>3</sub><sup>-</sup> in the pine forest ( $P = 0.005$ ).

in the old-growth forest than in the two younger forests. Our results from the control plots appeared to support this prediction. Average DON concentration in leachate collected below the major rooting zones in the old-growth forest was 1.8 and 2.3 times those in the respective pine and mixed forests. Correspondingly, DON flux in the old-growth forest was 2.0 and 2.6 times those in the pine and mixed forests (Table 1).

Our results showed that under ambient conditions DON concentrations and fluxes in soil leachates in the three study forests ranged from 1.4 to 3.2 mg N l<sup>-1</sup> and from 6.5 to 16.9 kg N ha<sup>-1</sup> y<sup>-1</sup>. Nitrogen deposition in this year (2005), measured as throughfall TDN input, ranged from 37.6 kg N ha<sup>-1</sup> in the mixed forest and 52.1 kg N ha<sup>-1</sup> in the old-growth forest, with DON input from 14.6 to 20.1 kg ha<sup>-1</sup> (Table 1). A similar amount of N deposition was found in bulk precipitation; bulk TDN input was 49.5 kg ha<sup>-1</sup>, including 17.8 kg ha<sup>-1</sup> as DON (Fang and others 2008). Much lower DON concentrations (<0.3 mg N l<sup>-1</sup>) in soil leachates (from 15 to 80 cm deep soil) were estimated from three humid tropical forests in northern Thailand, where low precipitation DIN input of 3.1 kg N ha<sup>-1</sup> y<sup>-1</sup> was observed (Möller and others 2005). Similarly, much lower DON concentrations (<0.2 mg N l<sup>-1</sup>) in soil water (at similar soil depth as ours) were found in the Hawaiian wet tropical forests with a broad range of stand maturity (Hedin and others 2003; Lohse and Matson 2005). In the old-growth forests in another wet tropical area (the

Atlantic Zone of Costa Rica, Central America), a slightly higher DON flux (3 kg N ha<sup>-1</sup> y<sup>-1</sup>) was found in soil solution collected at the same soil depth as our study (20 cm), where a moderate DIN deposition was measured in throughfall (17 kg N ha<sup>-1</sup> y<sup>-1</sup>, Schwendenmann and Veldkamp 2005). Michalzik and others (2001) summarized that annual DON fluxes in B-horizon soil solution from temperate forests were generally less than 8 kg N ha<sup>-1</sup>. The preliminary sampling in a small stream draining our old-growth forest pointed to a lower DON loss (DON and DIN exports of 4.3 and 12.9 kg N ha<sup>-1</sup> y<sup>-1</sup>, respectively, M. Yoh, unpublished data) than in soil leachate measured in this study, but that stream export was still higher than reported for most temperate forest zones (0.2–3.5 kg N ha<sup>-1</sup> y<sup>-1</sup>, Goodale and others 2000; Perakis and Hedin 2002) and for tropical Thailand (0.5–0.8 kg N ha<sup>-1</sup> y<sup>-1</sup>, Möller and others 2005). Our results suggest that DON loss from our forests might have been elevated by the high regional ambient N deposition (up to 50 kg N ha<sup>-1</sup> y<sup>-1</sup> in throughfall) in the past 15 years. This speculation is supported by the consistent and large increase in DON leaching observed in all of our three forests following experimental N applications. DON leaching was 35–162% higher in the N-addition plots than in the controls and the increased DON leaching comprised 4–17% of the annual experimental N additions (Table 1). The regression analysis showed that DON leaching responded positively to increasing DIN inputs (Figure 3).





**Figure 3.** Relationships between DON leaching (**A**) and NO<sub>3</sub><sup>-</sup> leaching (**B**) versus DIN input, and DON leaching versus NO<sub>3</sub><sup>-</sup> (**C**) and DOC leaching (**D**) in the respective pine, mixed, and old-growth forests: **A**  $P = 0.05, 0.095,$  and  $0.061,$   $R^2 = 0.44, 0.35,$  and  $0.34$  in the pine, mixed, and old-growth forests, respectively; **B**  $P = 0.001, 0.086,$  and  $0.025,$   $R^2 = 0.83, 0.36,$  and  $0.44;$  **C**  $P = 0.002, 0.014,$  and  $<0.001,$   $R^2 = 0.76, 0.60,$  and  $0.83;$  **D**  $P = 0.008,$   $R^2 = 0.66$  in the pine forest; the relationships between soil leachate DOC and DON fluxes were not significant in the mixed ( $P = 0.24$ ) and old-growth forests ( $P = 0.44$ ). DIN input was the sum of ambient throughfall DIN in the study year (Fang and others 2008) and experimental N addition.

Our results are in agreement with reports from two long-term N addition experiments in temperate forests in North America, where large and persistent increases in DON concentration in both the forest floor (McDowell and others 2004) and mineral soil (Magill and others 2000, 2004; Pregitzer and others 2004) have been shown following N additions. At the Harvard Forest, NH<sub>4</sub>NO<sub>3</sub> treatment had a statistically significant effect on DON concentration in forest floor leachate in both pine and hardwood stands and average DON fluxes (27 kg N ha<sup>-1</sup> y<sup>-1</sup>) in the high-N plots (annual addition of 150 kg N ha<sup>-1</sup> y<sup>-1</sup>) were triple those of the controls (McDowell and others 2004). In the soil solution below the rooting zone, DON concentration showed a delay in response, but the treatment effect became significant 9 years after the experiment commenced (Magill and others 2000, 2004). In the Great Lakes region, USA, DON fluxes below the rooting zone were elevated by 3.2 times over 8 years of experimental NO<sub>3</sub><sup>-</sup> additions (30 kg N ha<sup>-1</sup> y<sup>-1</sup>) in four northern hardwood

forests located along a 500-km gradient, with the effect becoming more pronounced over time (Pregitzer and others 2004). However, the quantities of DON loss (10–28 kg N ha<sup>-1</sup> y<sup>-1</sup>) in soil leachate in N-treated plots of our three forests in subtropical China were much higher than those of N-fertilized Michigan forests, where after 8 years of experimental NO<sub>3</sub><sup>-</sup> additions, absolute DON fluxes still remained at relatively low levels of 1–5 kg N ha<sup>-1</sup> y<sup>-1</sup> (Pregitzer and others 2004). The DON responses observed in our study are comparable to the floor leachate DON responses induced by long-term (15 years) and high dose (150 kg N ha<sup>-1</sup> y<sup>-1</sup>) N additions in the Harvard Forest (McDowell and others 2004) but higher than the response in the mineral soil solution that only occurred after 9 years of N treatment (Magill and others 2000, 2004). These results appear to confirm our initial hypothesis that DON production and loss would respond quickly to experimental N additions in a warm and humid climate. An increase of 150–900% in DON loss (from 0.31–0.99 kg N ha<sup>-1</sup> in

unfertilized plots to 1.8–4.2 kg N ha<sup>-1</sup> in fertilized plot) in the Hawaiian wet tropical forests was also found following short- or long-term N applications (Lohse and Matson 2005), but that DON increase was much smaller than the concurrent increase in NO<sub>3</sub><sup>-</sup> loss, such that NO<sub>3</sub><sup>-</sup> was the predominant form of N leaching loss in fertilized plots.

Under N-addition we found that the strongest response was not in the mature old-growth forest with more advanced initial N-saturation, but instead in the young pine forest (Figure 3, Table 1). The increase in DON fluxes in N-addition plots (compared to control) were 102–162% in the pine forest, compared to 59–61% and 35–69% in the mixed and old-growth forests, respectively. As forest types are not truly replicated, further research is required to address forest differences in response to N addition. However, the regression analysis showed that DON leaching was more related to NO<sub>3</sub><sup>-</sup> leaching than to DIN inputs (Figure 3). This result indicated that the magnitude of DON response may rely on the response of NO<sub>3</sub><sup>-</sup>.

There are an increasing number of studies showing that DON accounts for the majority of total N export from unpolluted forests and forests in areas with low atmospheric N input (Perakis and Hedin 2002; Gundersen and others 2006; Pellerin and others 2006). But ecosystem N loss as DIN is believed to be the predominant pathway following elevated N input. Some researchers thus suggest that DON loss may change little once a threshold N input has been exceeded and the loss of the highly mobile NO<sub>3</sub><sup>-</sup> would ultimately approach unity with input as abiotic and biotic compartments are saturated by chronic N exposure (Hedin and others 1995; Brookshire and others 2007). Our data (Table 1) together with the data from the N-fertilization studies in temperate forests (Gundersen and others 1998; McDowell and others 2004; Pregitzer and others 2004) generally support this proportional decline trend of DON with increasing N inputs. However, it is important to point out that a large absolute DON flux persisted or even increased in ecosystems receiving large inorganic N input, and that in the present study DON:TDN ratios remained above 20% even under the highest dose of N fertilization of 150 kg N ha<sup>-1</sup> y<sup>-1</sup> (Table 1). These results indicate that DON is important as a N-export mechanism and in balancing N budgets in both N-limited and N-saturated ecosystems. Additional research is needed to determine if this is a general pattern in forests receiving high inorganic N inputs and whether the DON fraction will go below 20% after a longer period of N treatment.

## Possible Mechanisms for Increased DON Loss under Elevated DIN Input

Several mechanisms may have simultaneously contributed to the increased DON loss from forests receiving high N input. We introduce a new conceptual model and discuss five potential mechanisms that most likely explain the consistent and large DON loss in our study (Figure 4). Elevated atmospheric N deposition has been proposed to increase the overall N cycling in forests (Aber and others 1998). This may occur in two ways, firstly by stimulating the decomposition and mineralization leading to the production of more DOM without much change in its C:N ratio (that is, concurrent increases of DOC and DON, mechanism 1, Figure 4), secondly by releasing N-rich products from N-enriched SOM during the mineralization process, leading to a decrease in the DOM C:N ratio (that is, minimal change in DOC leaching, mechanism 2, Figure 4). In the temperate forests studied by Pregitzer and others (2004), the first pathway seems to dominate with concurrent increases of DOC and DON. This pathway may also have occurred in our pine forest, whereas in our other two forests, almost no increase of DOC combined with a decrease in the C:N ratio of DOM (Figure 2) points to the dominance of the second pathway.

The SOM could be enriched in N directly via inorganic N incorporation through microbial immobilization, abiotic incorporation, and mycorrhizal assimilation (Aber and others 1998; Dail and others, 2001; Davidson and others 2003; Colman and others 2007). Microbial immobilization is frequently mentioned as the primary process for

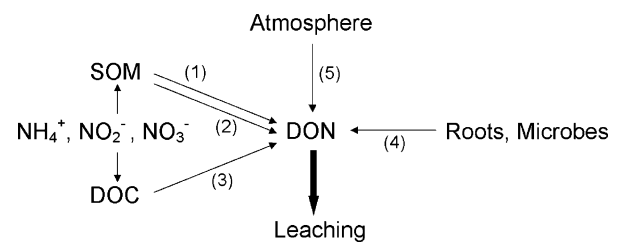


Figure 4. A conceptual model of DON biogeochemistry in forest ecosystems with increasing inorganic N availability: (1) enhanced decomposition and mineralization producing more DOM at an unchanged C:N ratio; (2) increased rates of depolymerization from N-enriched SOM (directly via incorporating inorganic N to SOM or indirectly via improved SOM quality), producing low C:N ratio DOM; (3) DON re-synthesis, with inorganic N reacting with DOC to produce N-enriched DOM (that is, with lower C:N ratio); (4) increased root exudation and microbe detritus; and (5) elevated atmospheric DON deposition.

incorporating mineral N into SOM, and mycorrhizal assimilation has been proposed to be an important mechanism for the efficient N retention in soils because mycorrhizae can use plant photosynthate as a carbon source (Aber and others 1998). Direct abiotic reactions of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  with SOM (abiotic incorporation) remain controversial (Aber and others 1998; Davidson and others 2003; Colman and others 2007). In contrast, nitrite ( $\text{NO}_2^-$ ), a chemically reactive intermediate of both nitrification and denitrification (Robertson and Groffman 2007), has been demonstrated to react quickly with SOM, even under abiotic conditions, and subsequently form DON (Dail and others 2001; Fitzhugh and others 2003). A consequence of N-saturation is the shift of N biogeochemistry from organic N or  $\text{NH}_4^+$  dominated states toward a  $\text{NO}_3^-$  dominated state (Aber and others 1998; Gundersen and others 1998; Zhu and Carreiro 2004). The large quantity of  $\text{NO}_3^-$  in an N-saturated system can create a flux of  $\text{NO}_2^-$ , and thereby a potential pathway for DON formation. In tropical forests and other warm and humid areas, such as where our study sites are located, the production of  $\text{NO}_2^-$  through both nitrification and denitrification could be high although the direct measurement of  $\text{NO}_2^-$  is difficult due to its chemically reactive nature (Davidson and others 1993). Our results showed that DON loss increased significantly with increasing DIN input, and the response of DON generally followed that of  $\text{NO}_3^-$  (Figure 3), suggesting that high  $\text{NO}_3^-$  availability could contribute significantly to N-enriched DOM. In the control plots of all three forests studied here, DOC:DON ratios were comparable to the C:N ratios of SOM; experimental N additions led to significant decreases of the C:N ratios of DOM (Figure 2). On the other hand, SOM could also become N enriched indirectly through improved quality of above- and belowground litter inputs (Aber and others 1998).

Of the two pathways discussed above, both fit the current shifting paradigm of N mineralization. Schimel and Bennett (2004) argued that the production of soluble organic N was the limiting step of N mineralization, not ammonification. Fast depolymerization of SOM compounds in the first step of mineralization produces N-rich DOM (amino acids or other N monomers) that may be leached. In our pine forest net N mineralization in the top 10 cm of soil was substantially elevated after 2 years of experimental N additions, and the DON loss reported in this paper (Table 1) was positively correlated with the concentration of extractable soil organic N (Fang and others unpublished data), supporting these mechanisms

of elevated DON loss when forests are moving toward N saturation.

The direct reaction of inorganic N and DOC to form N-enriched DOM (mechanism 3, Figure 4) is likely to occur in N-saturated soils due to a large flux of DIN, but it needs to be demonstrated by direct experimental evidence. It is also likely that in seasonally dry forests like those studied here, added N may be incorporated into microbial biomass and then desiccated during the dry season. The biomass lysed is then flushed out at the beginning of the wet season (mechanism 4, Figure 4). Increased plant N uptake has been observed as N limitation alleviates, which in turn could lead to more N-rich root exudations. Great increases in DON found at the beginning and end of the rainy season in our forests appear to support this speculation (Figure 1D–F).

The high concentrations of throughfall DON input may be a fifth and direct source of DON export at our sites (mechanism 5, Figure 4). It has been estimated that DON input as high as 15–20 kg N ha<sup>-1</sup> y<sup>-1</sup> could be expected in our forests (Fang and others 2008). However, a significant relationship between monthly DON leaching and throughfall DON input was only found in one of the forests (the mixed forest) despite the occurrence of heavy rains in the region, which could favor quick loss (Fang and others 2008). Neff and others (2002) suggested that DON deposition in polluted regions involved complicated atmospheric chemical reactions; our study here suggests that complicated biogeochemical reactions in the soil could generate additional DON, making DON leaching export a significant pathway of N loss in N-saturated ecosystems.

## CONCLUSIONS

The present study examined DON biogeochemistry in N-saturated forest soils under a warm and humid climate. Our data showed that DON leaching was substantially elevated by increased DIN inputs in all three subtropical forests in southern China, although the magnitude of response was forest-type dependent. DON accounted for a large fraction of total dissolved N loss in leaching under high N input (23–38%); the absolute DON fluxes measured in our sites were much higher than those reported from temperate forests receiving comparable N inputs. This result highlights that DON is likely to be a significant pathway of N loss from N-saturated forests and needs to be taken into account in balancing the N budget. Increased DON leaching may be the combined result of several

factors, but we cannot yet separate their respective contributions at the present time. We suggest that biological and abiotic incorporation of excessive  $\text{NO}_3^-$  into SOM could produce N-enriched DOM thereby elevating DON loss without substantially altering DOC loss. In addition, enhanced DON production due to rapid N cycling and direct DON input from atmospheric deposition are also likely.

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