Long-term nitrogen addition decreases carbon leaching in a nitrogen-rich forest ecosystem

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Abstract

Dissolved organic carbon (DOC) plays a critical role in the carbon (C) cycle of forest soils, and has been recently connected with global increases in nitrogen (N) deposition. Most studies on effects of elevated N deposition on DOC have been carried out in N-limited temperate regions, with far fewer data available from N-rich ecosystems, especially in the context of chronically elevated N deposition. Furthermore, mechanisms for excess N-induced changes of DOC dynamics have been suggested to be different between the two kinds of ecosystems, because of the different ecosystem N status. The purpose of this study was to experimentally examine how long-term N addition affects DOC dynamics below the primary rooting zones (the upper 20 cm soils) in typically N-rich lowland tropical forests. We have a primary assumption that long-term continuous N addition minimally affects DOC concentrations and effluxes in N-rich tropical forests. Experimental N addition was administered at the following levels: 0, 50, 100 and 150 kg N ha^{-1} yr^{-1}, respectively. Results showed that seven years of N addition significantly decreased DOC concentrations in soil solution, and chemo-physical controls (solution acidity change and soil sorption) rather than biological controls may mainly account for the decreases, in contrast to other forests. We further found that N addition greatly decreased annual DOC effluxes from the primary rooting zone and increased water-extractable DOC in soils. Our results suggest that long-term N deposition could increase soil C sequestration in the upper soils by decreasing DOC efflux from that layer in N-rich ecosystems, a novel mechanism for continued accumulation of soil C in old-growth forests.

Key words: Nitrogen deposition; Nitrogen saturation; N-rich; DOC efflux; Carbon cycle; Carbon sequestration; Soil solution; Tropical forest; Acidification

1 Introduction

Terrestrial ecosystem carbon (C) cycling and storage are a global concern in the context of increasing atmospheric deposition of N in the biosphere, especially in recent decades (Schindler and Bayley, 1993; Nadelhoffer *et al*., 1999; Galloway *et al*., 2004; LeBauer and Treseder, 2008; Hyvönen *et al*., 2008). Although it is generally known that N deposition can significantly alter terrestrial ecosystem C cycle, most studies on the responses of ecosystem C cycling to N enrichment focused on net primary productivity (NPP), net ecosystem productivity, net ecosystem CO_2 exchange, and labile pools of C (LeBauer and Treseder, 2008; Hyvönen et al., 2008; de Vries et al., 2009; Liu and Greaver, 2010; Thomas et al., 2010). In contrast, effects of N on dissolved organic C (DOC) have received less attention, likely because these effluxes are small relative to the C fluxes associated with primary productivity or heterotrophic respiration in terrestrial systems (Kalbitz et al., 2000; Neff and Asner, 2001). However, the dynamics of DOC are receiving increased attention, considering their essential links in the bio-, hydro- and pedosphere (Kalbitz et al., 2000) and their central importance in soil-forming processes and carbon sequestration via DOC mobilization and transport for both temperate and tropical soils (McDowell, 1998; Monteith et al., 2007; Cusack et al., 2010; Liu & Greaver, 2010; Kindler et al., 2011).

Forest soils play a key role in the global C cycle (Lal, 2005). To explore the importance of DOC effluxes under elevated N deposition in forest ecosystems, ecologists have conducted such studies by the methods of simulating N deposition or using natural N deposition gradients (Evans et al., 2008; Sleutel et al., 2009). Until now, these studies are limited to DOC dynamics (e.g., concentrations or effluxes), and have not been linked to the possible C sequestration induced by N deposition in ecosystems. Meanwhile, these studies are mostly focused in temperate regions, especially in North American and Europe, where ecosystems commonly belong to glaciated landscapes and are N-limited under natural conditions (e.g. Vitousek and Howarth, 1991; Aber et al., 1998, 2003; Magill et al., 2004). These studies often find that DOC concentration in soil solution increases with elevated N deposition (Yano et al., 2000; McDowell et al., 2004; Pregitzer et al., 2004; Adams et al., 2005; Findlay, 2005; Sleutel et al., 2009; Rappe-George et al., 2012).

Tropical forest ecosystems, which store approximately 13% of global soil C, contribute greatly to the global C cycle; thus, even relatively small fluctuations in C cycling can have global consequences (Post et al., 1982; Phillips et al., 1998; Findlay, 2005; Townsend et al., 2011). In contrast to their temperate and boreal counterparts, many lowland tropical forests are typically N-rich ecosystems, with high soil N availability, rapid rates of N cycling, and the lack of N limitation to NPP (Vitousek & Sanford, 1986; Matson et al., 1999; Hedin et al. 2009; Wright et al., 2011; Brookshire et al., 2012). However, our understanding of how N additions control DOC dynamics in these N-rich ecosystems remains far from complete.

The purpose of this study was to examine the effects of how long-term (7 yr) experimental addition of N affects DOC dynamics in the N-rich tropical forests. In 2002, we established long-term N deposition research plots in typical N-rich lowland tropical mature forests of Southern China (Mo et al., 2006. 2008; Fang et al., 2009; Lu et al., 2010), where atmospheric N deposition rates are commonly > 19 kg N ha^{-1} yr^{-1}, and are expected to increase greatly in the future due to the rapid development of agricultural and industrial activities (Zhou and Yan, 2001; Galloway et al., 2004; Lü and Tian, 2007; Liu et al., 2011). Because soil solution chemistry can be considered as a sensitive indicator of biogeochemical processes within forest stands, responding quickly to disturbances or stresses such as excess N (e.g. McDowell et al., 2004;

Pregitzer et al., 2004; Michel et al., 2006; Gilliam and Adams, 2011), we mainly focused our study on the response of soil solution chemistry to N addition. Earlier measurements in these forests have indicated no changes in DOC dynamics in response to short-term (1 to 2 years) N deposition treatment (Fang et al., 2009). In the present study, we expected to find that long-term continuous N addition to N-rich tropical forests has minimal effect on DOC concentrations and effluxes, because highly weathered tropical soils commonly have high levels of N availability and rapid N cycling (Martinelli et al. 1999; Vitousek and Sanford, 1986; Fang et al., 2009). At the same time, we assumed that mechanisms for N-addition induced changes of DOC dynamics may be different from those of N-limited temperate forests, because of the different N status.

2 Materials and methods

2.1 Study Site

We carried out our work in the Dinghushan Biosphere Reserve (DBR). This site is part of the UNESCO/MAB network and is within the Guangdong Province of southern China (112º10' E, 23º10' N). The DBR extends approximately 1,200 ha within the subtropical/tropical moist forest life zone. It was established in 1950 for protection of remnant of undisturbed monsoon evergreen broadleaf forests in the lower subtropics, being the first National Natural Reserve in China in 1956. The monsoon climate of this site averages 1927 mm precipitation per years with approximately 75% occurring between March and August, and 6% between December and February (Huang & Fan, 1982). Relative humidity averages 80% throughout the year. Mean annual temperature is 21.0 C, ranging from mean coldest in January (12.6 C) and hottest in July (28.0 C). Currently, the region surrounding DBR experiences high rates of atmospheric N deposition (21-38 kg N ha^{-1} yr^{-1} as inorganic N in bulk precipitation) (Huang et al., 1994; Zhou and Yan, 2001; Fang et al., 2008). In 2004—2005 wet N deposition averaged ~33 kg N ha^{-1} y^{-1} (Fang et al. 2008).

We established the research site at DBR in 2002 between 250 and 300 m above sea level. According to ^{14}C measurement, forest stands have been protected from direct human disturbance for > 400 years (Shen et al., 1999). These support a rich assemblage of plant species, most of which are evergreen tree species native to the tropics and subtropics. These include *Castanopsis chinensis* Hance, *Schima superba* Chardn. & Champ., *Cryptocarya chinensis* (Hance) Hemsl., *Cryptocarya concinna* Hance, *Machilus chinensis* (Champ. Ex Benth.) Hemsl., and *Syzygium rehderianum* Merr. & Perry (Cao et al., 2002). Canopy closure is typically above 95% (Lu et al., 2010). Soils are oxisols (lateritic red earths) formed from sandstone approximately 30 cm to 70 cm in depth.

2.2 Experimental treatments

The experiments involving N amendments were established in July 2003 (Mo et al., 2006), with four N addition rates used: Control (0 N added), Low-N (50 kg N ha^{-1} yr^{-1}), Medium-N (100 kg N ha^{-1} yr^{-1}) and High-N (150 kg N ha^{-1} yr^{-1}), which were based on the present atmospheric N deposition rate and the expected increase in the future due to the rapid development of agricultural and industrial activities (Galloway et al., 2004; Lü and Tian, 2007). Considering that any effects of chronic low level N addition are likely to be similar in direction, if not magnitude, to the short-term effects of high rates of N addition (e.g. Báez et al. 2007; Clark & Tilman 2008; Lu e al., 2010), results from our present concentration gradients could be as a prediction for the future changes. A 10 m wide buffer strip surrounded each of 12 10-m x 20-m plots, with plots and treatments replicated in triplicate and randomly located. A hand-applied NH_4NO_3 solution was added each month to the forest floor of each plot as 12 equal, monthly applications per year. Fertilizer was weighed and mixed with 20 L of water (equivalent of 0.1 mm rainfall), with solution added via backpack sprayer below the canopy. Two passes were made across each plot to ensure an even distribution of fertilizer. Control plots received an equivalent volume of deionized H_2O.

2.3 Field water sampling and laboratory analysis

Precipitation and air temperature were monitored in an open area adjacent to the study plots. The data used in this study were from the weather station in the reserve (Appendix 1).

Soil solution was collected at a 20 cm depth, a depth which represents the primary rooting zone, and containing >70% of fine root biomass and 68% of total root biomass (Liao et al., 1993; Wen et al., 1999). Soil solution was sampled with two replicate zero tension tray lysimeters (755 cm^2 per tray) per plot, which were installed in April/May 2003 (i.e., 3-4 months prior to our experiment). Each lysimeter was connected with Tygon tubing to a 10-L bottle.

Soil solution samples were taken after each rain event (particularly after heavy rainstorms) from July 2009 to June 2010. Soil solution volume was recorded and composited within a plot on the data of collection. All collectors were washed with deionized H_2O immediately following each collection.

Within 24 to 48 hr of field collection, soil solution samples were filtered through 0.45 mm micron filters in the laboratory, and then stored in plastic bottles at 4°C until chemical analysis, which included NH_4^+-N, NO_3^--N, DOC, and pH. A Shimadzu

TOC-VCSH Total Organic Carbon analyzer was used to determine DOC, with samples combusted at 680°C via platinum catalyst and CO_2 determined with a non-dispersive infrared (NDIR) detector. Samples were analyzed for dissolved inorganic nitrogen (NH_4^+-N and NO_3^--N) using a Lachat QC8000 Flow Injection Analyzer.

2.4 Field soil sampling and laboratory analysis

Samples of mineral soil were collected in August 2009 with a 5-cm diameter corer at 0–10 and 10–20 cm depths. From 0–10 cm, cores were taken beneath the loose litter layer (Oi) and comprised Oe and Oa horizon plus mineral soil to a total depth of 10 cm. Following this, the corer was driven to a 20 cm depth for sample collection. Sampling in each plot took place in three randomly-selected locations

In the laboratory, roots and stones were removed by sieving soil to pass a 2-mm screen; sieved soils mixed thoroughly by hand. For water-extractable DOC (WDOC) measurements, one 10 g sub-sample from each sample was extracted with 50 ml of deionized H_2O for 30 min and filtered through 0.45 µm cellulose–acetate filters, as modified from (Hagedorn et al., 2002). Water-extractable DOC was determined with a Shimadzu TOC analyzer as previously described. Other subsamples were air-dried and used to measure pH (soil:water = 1:2.5) and nutrient content. Total C (total soil organic C) was measured via titration with Fe^{2+} solution following dichromate oxidation (Liu et al., 1996). Total N was determined by determination of NH_4^+ following semi-micro Kjeldahl digestion (Liu et al., 1996). Exchangeable Fe and Al were extracted with 0.1mol/L $BaCl_2$ (50:1, solution:soil). Subsamples of soil were oven-dried at 105°C to a constant weight (at least 24 hr) to allow reporting soil results on an oven-dry basis.

2.5 Field litterfall sampling

Two 1-m x 1-m litter traps with a 1-mm mesh size were placed randomly in each plot at an approximate 0.5-m height above ground surface. Traps were emptied each month during the year, with litterfall separated into three components: leaves, small woody material (branches and bark), and miscellaneous (mainly reproductive parts).

2.6 Data analyses

Monthly and annual C effluxes from the primary rooting zone for each plot were calculated by multiplying DOC concentrations of soil leachate by the recorded water volume for each sample collection and then summed appropriately. We calculated mean values per month for NH_4^+-N, NO_3^--N, DOC and pH in water samples for further analysis. Effects of N treatments on soil solution chemistry (NH_4^+-N, NO_3^--N, DOC and pH) and litterfall during the study period were assessed with repeated measure analysis of variance (ANOVA). One-way ANOVA with Tukey's honestly significantly different (Tukey's HSD) test was used to test N treatment effects on concentrations of NH_4^+-N and NO_3^--N, pH, and annual DOC effluxes for the whole study period. One-way ANOVA with Tukey's HSD test was also employed to identify N treatment effects on soil properties (soil pH, concentrations of total C and N, C/N ratios, and extractable Fe and Al) and WDOC. Extractable Fe and Al pools were estimated by multiplying extractable concentrations by soil bulk density, which were taken from the Dinghushan research station. We conducted the planned contrast analysis to test differences between Control plots and N-treatment plots.

We also used general linear models to analyze relationships between DOC concentrations and pH for soil solution sampled at 20 cm depth in all plots during the study period. Linear regression analysis was also used to examine the relationship between mean DOC of cm soil solution at 20 cm depth and extractable Fe and Al pools in the upper 20 cm soil, respectively. All analyses were conducted using SPSS 14.0 for Windows® (SPSS, Chicago, IL, USA), with significant differences set with $P < 0.05$, unless otherwise stated.

3 Results

During the study period (July 2009 to June 2010), the total precipitation was 1992 mm, most falling during the March to August wet season (Appendix. 1). Mean monthly precipitation in wet season (245 mm) nearly three times that of dry season (88 mm). Mean monthly temperature was 22.2 °C. Total wet N deposition was 34.4 kg N ha^{-1}, with 18.2 kg ha^{-1} dissolved inorganic N (7.7 kg ha^{-1} NO_3^--N and 10.5 kg ha^{-1} NH_4^+-N, respectively) and 16.2 kg ha^{-1} dissolved organic N, respectively.

3.1 DOC concentration and effluxes

The repeated measures ANOVA revealed that N additions significantly decreased the DOC concentrations and DOC effluxes at 20 cm depth over the study period (df=3, F=21.4, P=0.001; df=3, F=6.8, P=0.02, respectively) (Figure 1a and c). There were also significant interaction effects between treatment and time (months) on DOC concentrations and effluxes (df=33, F=3.6, P<0.001; df=33, F=2.1, P=0.006, respectively). For DOC concentrations, the decreased trends were more pronounced in Medium-N and High-N plots than that of Low-N plots, and relative measures showed they decreased by 15%, 28% and 31% in the Low-N, Medium-N and High-N plots, respectively, relative to that of the Control plots over the whole year (Figure 1b). For DOC effluxes, they decreased by 44%, 34% and 18% in the Low-N, Medium-N, and High-N plots, respectively (Figure 1d).

Mean DOC concentrations in the Medium-N and High-N treatments were significantly lower than that of the Controls (*P*<0.05; Table 1). Further analysis showed that N additions decreased annual DOC effluxes at 20 cm, especially in the Low-N and Medium-N plots, where the decreases were significant (*P*<0.05; Table 1). Planned contrast analysis showed that there were significant N-treatment effects for both mean DOC concentrations and annual DOC effluxes. The annual DOC effluxes were 99.6, 63.6, 61.0, and 79.1 kg C ha^{-1} yr^{-1} in the Control, Low-N, Medium-N and High-N plots, respectively.

3.2 NO_3^--N, NH_4^+-N and pH in soil solution

The concentrations of NO_3^--N in N-treatment plots were generally higher than that of the Controls (Fig. 2a), and the mean concentrations of NO_3^--N across the whole study period increased significantly in N-treatment plots (*P*=0.099; Table 1), although repeated measures ANOVA revealed that N additions did not significantly increase concentrations of NO_3^--N (*df*=3, *F*=2.4, *P*=0.16).

Concentrations of NH_4^+-N (commonly less than 1 mg N L^{-1} as mean values for the whole period) were much lower than those of NO_3^--N at all plots (Figure 2b). There were no significant responses to N treatments across all plots and sampling times. This is further confirmed by the result of repeated measures ANOVA (*df*=3, *F*=1.4, *P*=0.34). In addition, the mean concentrations of NH_4^+-N across the whole study period also showed no significant differences between N treatments and Controls (Table 1).

Repeated measures ANOVA showed that N additions significantly decreased soil solution pH it at 20 cm depth (*df*=3, *F*=42, *P*<0.001). Similarly, across the whole study period, N treatments changed significantly (*P*=0.001) the mean values of soil solution pH, especially in the High-N plots (Table 1).

3.3 Soil chemistry and litterfall

Total soil N and C and extractable Al and Fe showed increasing trends with elevated N addition (Table 2). Total N increased by 8%, 12%, 17% in the Low-N, Medium-N, and High-N treatment plots, respectively, compared to the Controls; total C increased by 10%, 13%, 17% in the Low-N, Medium-N, and High-N treatment plots, respectively, compared to Controls. High-N treatments also showed marginally significant effects on total C (P=0.08), and significant effects on extractable Fe (P=0.03). Soil pH values decreased with increasing N treatment levels, especially in the Medium-N and High-N plots (P<0.1). Further analysis showed that there were significantly linear relationships between treatment levels and pH across all plots (R^2=0.54, N=12, P=0.006). There was no significant difference among treatments for soil C/N ratios. For WDOC, N additions increased their contents in this upper 0-20 cm soil, where the increases were significant under N treatments compared to the Controls (P=0.032; Table 2).Annual litterfall was not significantly different among treatments (Figure 3).

3.4 Relationships between DOC, extractable Fe and Al, and pH

Linear model analysis showed that DOC concentrations were significantly and positively correlated with pH (R^2=0.4; N=144; P<0.001) in soil solution across all sampling data (Figure 4). Meanwhile, extractable Al pool was not significantly correlated with mean DOC concentration in the soil solution at 20cm soils (R^2=0.004, N=12, P=0.84; Figure 5a), but extractable Fe pool exhibited significant and negative correlations with DOC (R^2=0.42, N=12, P=0.023) (Figure 5b).

4 Discussions

4.1 Effects of N addition on DOC leaching

Earlier measurements in the year 2005 at our site showed that N addition had no significant effects on soil solution DOC concentrations below the primary rooting zone (Fang et al., 2009). Current results, however, indicate that N treatments significantly decreased DOC concentrations in soil solution from this layer, suggesting that responses of DOC dynamics to N addition may be time-dependent in N-rich tropical forests. This rejects our initial hypothesis, and also contrasts with other studies in primarily N-limited ecosystems. In N-limited forests, increased N availability generally results in more DOC production and subsequent leaching (Pregitzer et al., 2004; Findlay, 2005; Adams et al., 2005; Sleutel et al., 2009). Smemo et al. (2007) reported that deposition added as $NaNO_3$ significantly increased soil solution DOC concentration and export from four different northern hardwood forests in the Great Lakes region. McDowell et al. (1998, 2004) found greater concentrations of DOC following the addition of 50-150 kg N ha^{-1} yr^{-1} as NH_4NO_3. Sleutel et al. (2009) provide additional evidence on higher concentrations of DOC in boreal forests of Belgium under historic high N deposition.

Biological mechanisms (balance between processes that produce and consume DOC) are often suggested to explain changes of DOC concentrations in leachate with elevated N addition in typically N-limited ecosystems (Neff and Asner, 2001; Knorr et al., 2005; Zak et al., 2008; Evans et al., 2008). Pregitzer et al. (2004) suggested that increases in DOC were primarily biologically driven, resulting from changes in production of organic substrates and processing within soil food webs. Bragazza et al. (2006) indicated that the increased release of DOC from litter peat was a consequence of enhanced enzymatic activity (e.g., phenol oxidase). In these studies, litterfall is the major source of DOC to the forest floor and thus deeper soil horizons with elevated N deposition (Currie et al., 1996; Magill and Aber, 2000; Park et al., 2002; Sleutel et al., 2009). Gundersen et al. (1998) showed a significant correlation between DOC concentration beneath the forest floor (Oa horizon) and litterfall amount. In a meta-analysis from multiple terrestrial ecosystems, Liu & Greaver (2010) found that N addition increased soil DOC concentration by an average of 18%, although soil respiration was not altered, suggesting C leaching loss may increase in N-limited ecosystems.

In our N-rich forest, however, there were no significant effects of N treatments on litterfall production (Mo et al., 2008; Figure 3); at the same time, there was no significant difference between N treatments in DOC dynamics in surface runoff (data not shown), suggesting that litterfall inputs may play a minor role in DOC production and subsequent fluxes into deeper soils under N treatments. Further studies showed that N addition significantly inhibited litter decomposition and decreased soil respiration in this forest (Mo et al., 2006, 2008). In addition, C mineralization, which is the conversion from the organic C form to inorganic compound as a result of decomposition reactions (Carter and Gregorich, 2008), is suggested to result in high absolute loss of DOC (Chantigny et al., 1999; Huang and Schoenau, 1998; Sjöberg et al., 2003). An incubation experiment this forest soils, however, showed that N addition decreased organic C mineralization (Ouyang et al., 2008), indicating that elevated N inputs may contribute to soil DOC accretion in the deeper soil by decreasing DOC decomposition/consumption Our findings support this suggestion in that N addition greatly increased water-extractable DOC (Table 2), a finding also supported by other studies (e.g. Hagedorn et al., 2002; Sinsabaugh et al., 2004; Gallo et al., 2005). Increases in extractable DOC under experimental N additions are generally suggested to increase regional and global DOC effluxes from terrestrial ecosystems to aquatic ecosystems (Pregitzer et al., 2004; Findlay, 2005; Mo et al., 2008; Chapin et al., 2009). However, our results showed that N treatments decreased DOC concentrations in leachate solutions. Therefore, biological control mechanisms are unlikely responsible for declines in DOC in soil leachate of this N-rich tropical forest.

Indeed, as suggested by Neff and Asner (2001), physical controls may also play an important role in dominating DOC transformations in soils. Here, we propose that both changes in solution acidity and soil sorption dynamics play a dominant role in regulating DOC losses from N-rich ecosystems.

For example, acidity of soil solution may regulate the patterns of DOC responses. It has been recognized that the increase of soil solution pH (or acid neutralizing capacity) would lead to the net positive changes in DOC concentration by increasing DOC solubility in soil (Monteith et al., 2007; Evans et al., 2008). This was demonstrated by Evans et al. (2008) while reviewing field N addition experiments in Europe and North American. It has been widely accepted that high N deposition could accelerate soil acidification and have the potential to change the acidity of soil solution (Aber et al., 1989; Vitousek et al., 1997; Bowman et al., 2008; Van den Berg et al., 2008). In this study, we found that N treatments significantly decreased soil solution pH below the dominant rooting zone. Further analysis showed that there was a significant and positive relationship between soil solution pH and DOC concentration.

It should be noted that the effect of pH on DOC dynamics may be confounded with other mechanism related to soil properties, for example, sesquioxides in the mineral soil (Moore et al., 1992; Guggenberger, 1994; Michalzik et al., 2001). Thus, we suggest an alternative mechanism for our observations. Soils containing high concentrations of extractable Fe or/and Al exhibit the capacity to adsorb DOC as water percolates down through the soil profiles thereby decreasing DOC concentrations (Boudot et al., 1989; Guggenberger, 1994; Kaiser and Guggenberger, 2000; Sleutel et al., 2009). Corre et al. (2010) suggested that sorption by hydrous Al oxides could be an important reason for N -induced decreases of soil solution DOC. In our study, however, the extractable Al did not vary significantly among Controls and N-treatment plots, and there were no significant relationships between the extractable Al pool and mean DOC concentration (Table 2, Figure 4a). By contrast, N addition significantly increased extractable Fe. Also, there was a significant negative relationship between extractable Fe pool and mean DOC concentration at 20 cm soil solution (Figure 4b). Accordingly, it is possible that extractable Fe, rather than extractable Al, may play a key role in DOC adsorption in N-treatment plots after long-term N inputs. A better understand how Fe and Al oxides control DOC dynamics in tropical forests merits further study.

4.2 Effects of N addition on annual DOC effluxes

Annual DOC effluxes below the 20-cm rooting zones in our study ranged from 60-100 kg C ha^{-1} yr^{-1}, well within the range (30-139 kg C ha^{-1} yr^{-1}) reported for tropical forests by Aitkenhead and McDowell (2000). Our results demonstrated that long-term N addition decreased annual DOC effluxes from the primary rooting zones, especially in the Low-N and Medium-N plots. The decreases in annual DOC effluxes indicated that soils may accumulate much more DOC with elevated N addition, consistent with the significant increase of water-extractable DOC at 0-20 cm soil layer in N-treatment plots (Table 2), and suggesting that elevated N deposition might enhance soil C sequestration by decreasing DOC effluxes in N-rich forests.

Zhou et al. (2006) found that this old-growth forest could accumulate soil C (0–20cm depth) at about 610 kg C ha^{-1} yr^{-1} over the last two decades, but concluded that the reason for this accumulation was unclear. Our results showed that N-induced net C sequestration (via reduced DOC efflux, calculated by the difference between N-treatment plots and the Controls) was about 36, 39, 21 kg C ha^{-1} yr^{-1} in the Low-N, Medium-N, and High-N plots respectively, with a mean value of 32 kg C ha^{-1} yr^{-1} in N treatment plots, relative to controls (Table 1). In fact, we have found that N treatments significantly increased soil total C after long-term N addition (Table 2). Therefore, such decreases in annual DOC effluxes may explain, in part, this accumulation of soil C observed by Zhou et al. (2006) considering the high N deposition during the past decades in this region.

4.3 Implications

We have studied effects of long-term N additions on DOC dynamics of soil solution in N-rich lowland tropical forests under a warm and humid climate. Our results showed that long-term N additions significantly decreased DOC concentrations in soil solution of deeper soils. It was suggested that chemo-physical controls (solution acidity change and soil sorption) rather than biological controls could play a dominant role in regulating DOC losses from N-rich ecosystems, in contrast to that of N-limited ecosystems. We further found that N addition decreased greatly annual DOC effluxes below the primary rooting zones, and increased water-extractable DOC in soils. It is suggested that DOC constitutes an important carbon input efflux to forested mineral soils (Schwesig et al., 2003), and DOC adsorbed by soils may contribute to the stock of organic C accumulating during soil development (Qualls and Bridgham, 2005). Therefore, our results indicate that long-term N deposition could increase soil C sequestration in the upper soils by decreasing DOC efflux in N-rich forests, which may support a novel mechanism responsible for continuing to accumulate C in old-growth forests (Zhou et al., 2006; Luyssaert et al., 2008). Thus, this study may give us a new understanding on forests ecosystem C cycling and possible C sequestration, and also support data bases for model predictions in N-rich ecosystems, with the globalization of N deposition. Although our findings would be typical for other N-rich sites, however, our results and corresponding control mechanism should be further validated in various tropical ecosystems in the future with elevated N deposition.

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Tables

Table 1 Effects of N addition on average concentrations of DOC, NO_3^--N, NH_4^+-N, and pH, and annual DOC efflux in soil solutions below the primary rooting zones (0-20 cm soils) during the periods from July 2009 to June 2010. The different lowercase letters indicate significant differences at *P*<0.05 level, and no letters indicate no significant differences among N treatment levels, respectively (Tukey's HSD test); *Contrast Test* is conducted between N treatments and the Controls by using planned contrast analysis. Values are mean with S.E. in parentheses.

N treatments	DOC ($mg\ L^{-1}$)	DOC efflux kg C $ha^{-1}\ yr^{-1}$	NO_3^--N (mgL^{-1})	NH_4^+-N ($mg\ L^{-1}$)	pH
Control	23.96(2.18)a	99.61(2.63)a	9.74(0.92)	0.24(0.01)	3.81(0.01)a
Low-N	20.19(1.16)ab	63.62(6.25)b	11.26(0.40)	0.23(0.01)	3.78(0.01)a
Medium-N	17.10(0.92)b	60.99(8.87)b	11.74(0.82)	0.24(0.03)	3.78(0.00)a
High-N	14.98(0.46)b	79.06(3.73)ab	14.04(2.05)	0.45(0.20)	3.70(0.01)b
Contrast Test	*P*=0.003	*P*=0.002	*P*=0.099	*P*=0.58	*P*=0.001

Table 2 Responses of soil chemistry in the primary rooting zones (0-20 cm soils) to N addition in the lowland tropical forest of southern China in August, 2009. The different lowercase letters indicate significant differences at $P<0.05$ level, and no letters indicate no significant differences among N treatment levels, respectively (Tukey's HSD test). *Contrast Test* is conducted between N treatments and the Controls by using planned contrast analysis.

	N treatments				
Parameters	Control	Low-N	Medium-N	High-N	*Contrast Test*
Total N (mg g^{-1})	1.90(0.11)	2.05(0.08)	2.13(0.09)	2.22(0.06)	P=0.045
Total C (mg g^{-1})	21.88(0.40)	24.17(0.97)	24.82(0.50)	25.64(1.44)	P =0.023
C/N ratio	11.27(0.45)	11.53(0.83)	11.45(0.59)	11.29(0.44)	P =0.83
WDOC(mg Kg^{-1})	107.43(8.24)	160.92(25.55)	140.10(10.97)	179.20(20.30)	P =0.032
Al^{3+} (m mol kg^{-1})	30.50(1.31)	30.54(2.78)	31.49(1.66)	31.54(1.60)	P =0.67
Fe^{3+} (m mol kg^{-1})	0.12(0.013)a	0.17(0.018)ab	0.17(0.020)ab	0.20(0.010)b	P =0.012
pH (H_2O)	3.87(0.02)	3.84(0.00)	3.75(0.05)	3.75(0.04)	P =0.045

Notes: Total C means total soil organic carbon; WDOC means water-extracted dissolved organic carbon; Values are means with SE in parentheses.

Figure Legends

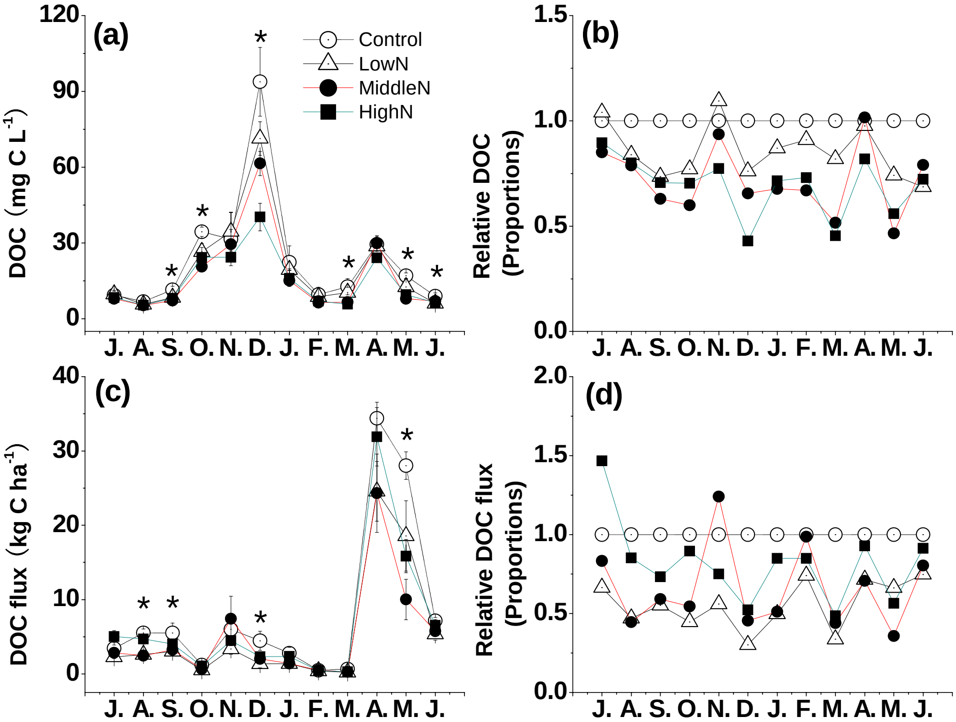
Figure 1 Responses of DOC concentration (a) and its relative concentration (b), and DOC efflux (c) and its relative efflux (d) to long-term N addition below the primary rooting zone in the lowland tropical forests of Southern China. Soil leachate data were available from July 2009 to June 2010. Notes: Asterisk (*) indicates that there are significant differences at $P<0.05$ level between N treatments and the Controls by using planned contrast analysis.

Figure 2 Responses of NO_3^--N (a), NH_4^+-N (b) and pH (c) dynamics to long-term N addition in soil solution below the dominant rooting zone in the lowland tropical forests of Southern China. Asterisk (*) indicates that there are significant differences at $P<0.05$ level between N treatments and the Controls by using planned contrast analysis.

Figure 3 Monthly dynamics of litterfall with elevated N addition in the lowland tropical forests of Southern China during the study period.

Figure 4 Relationships between DOC concentrations and pH in soil solutions across all plots during the study period. Notes: Triangles (Δ) indicate DOC concentration at control plots, and solid circles (●) indicate DOC concentration at N-treatments plots.

Figure 5 Relationships between mean DOC concentrations in soil solution during the study period and soil extractable Al and Fe pools in upper 0-20cm mineral soils.



754

755 **Figure 1**

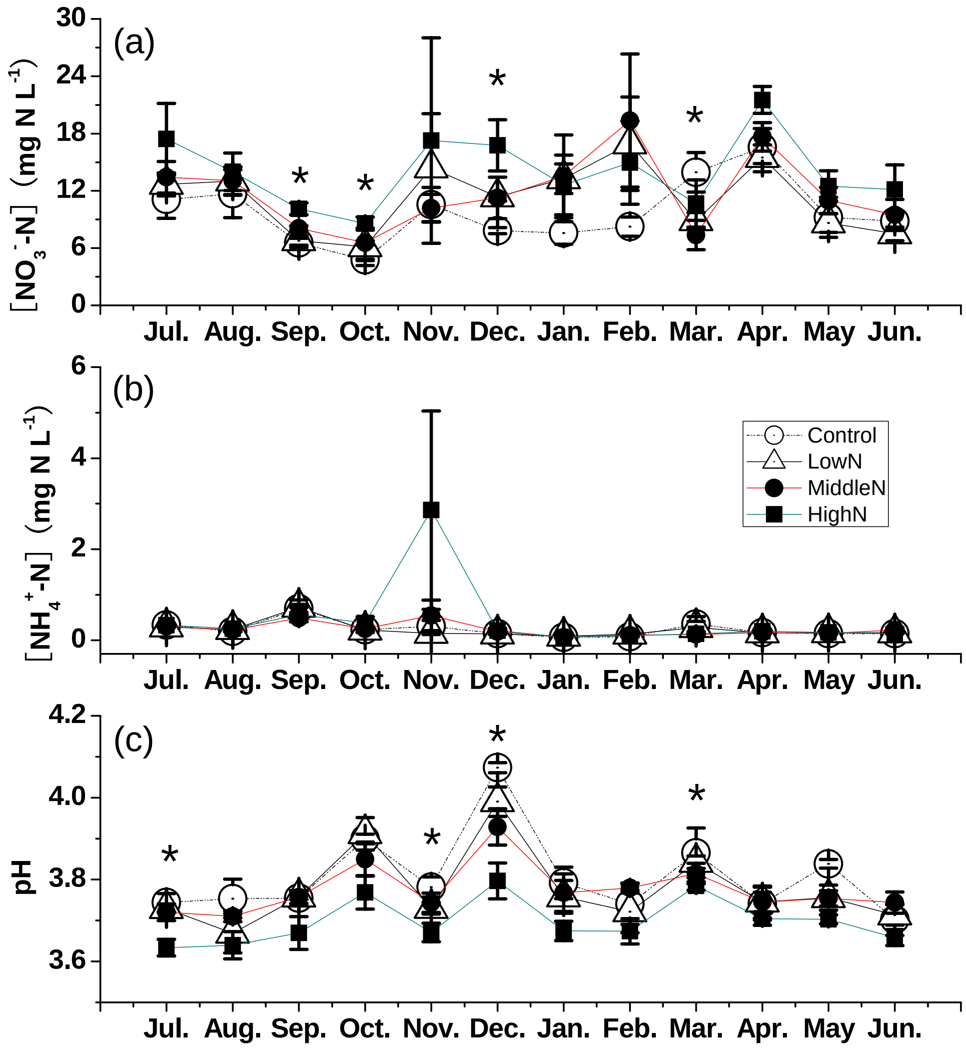

(a)
(b)
(c)
[NO3--N] (mg N L-1)
[NH4+-N] (mg N L-1)
pH
Jul. Aug. Sep. Oct. Nov. Dec. Jan. Feb. Mar. Apr. May Jun.
Control
LowN
MiddleN
HighN
*

Figure 2

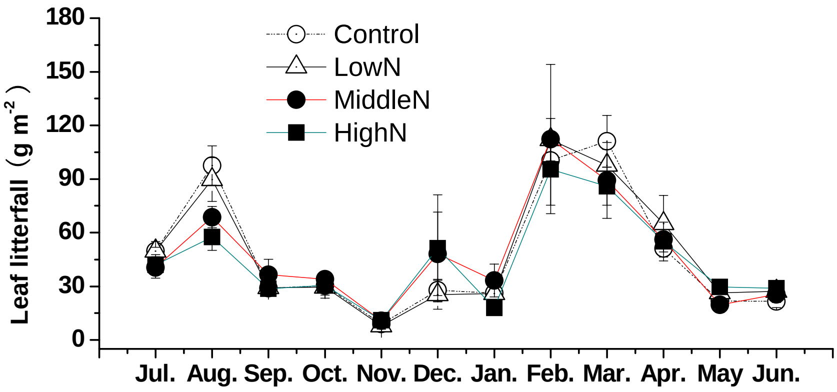


Figure 3

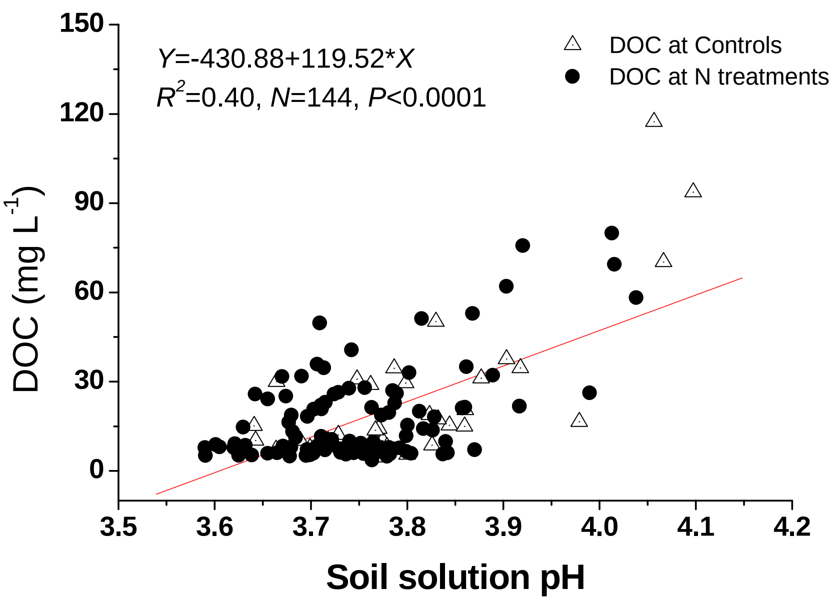


Figure 4

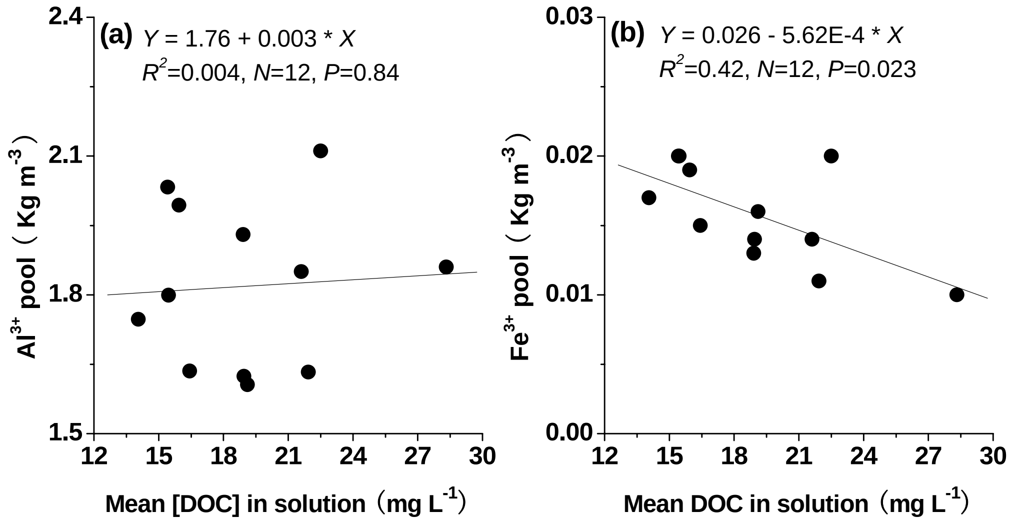
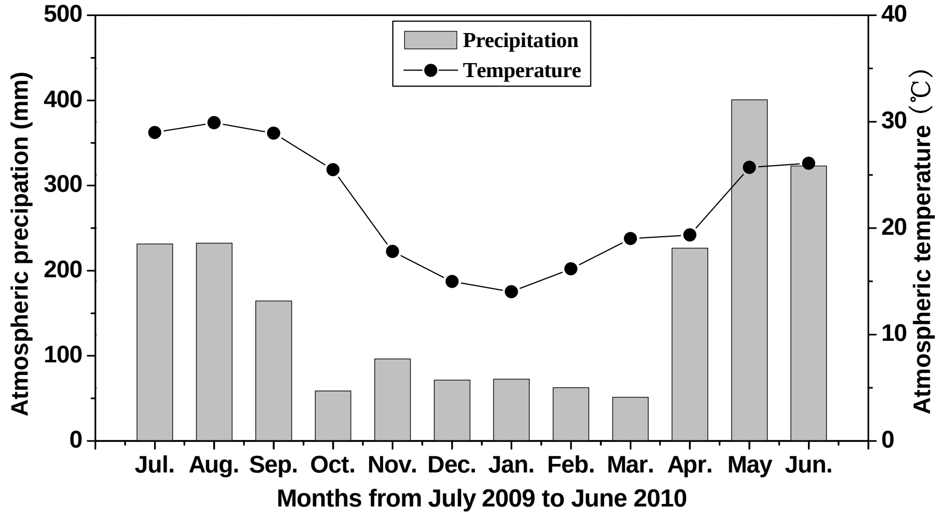


Figure 5



Appendix 1 Monthly precipitation and monthly mean air temperature at Dinghushan Biosphere Reserve, southern China, during this study period.