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Net Nitrogen Mineralization and Nitrification in Three Subtropical Forests of Southwestern China

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ABSTRACT

Nitrogen is a limiting nutrient for plant growth in many forest ecosystems; however, information on N mineralization and nitrification in subtropical forests is generally lacking. To determine the effect of forest site on N mineralization and nitrification and the key contributing factors under subtropical climate, we studied the seasonal patterns of soil N mineralization and nitrification with closed *in situ* core incubation method in a mixed evergreen broadleaf forest, a pure Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) forest, and a pure Zhennan (*Phoebe zhennan* S. Lee et F. N. Wei) forest in Dujiangyan, Sichuan province, southwestern China. Our results indicated that the rates and the seasonal patterns of net N mineralization and nitrification were similar among the three forest sites. The rates of both net N mineralization and nitrification were the highest in the winter season (December 2000 to April 2001), and negligible in spring and summer. The variability in net N mineralization and nitrification and the specific environmental contributing factors, however, varied in different forest sites. The overall similarity in N mineralization and nitrification rates suggests that regional climate condition is the most dominant controlling factor on N mineralization and nitrification. At local scales, however, biodiversity may play an important role in patterns of N mineralization and nitrification; higher biodiversity could lead to higher and more stable N mineralization and nitrification.

Keywords: net N mineralization, nitrification, Sichuan province, soil temperature, soil water content, subtropical forest, throughfall

INTRODUCTION

Nitrogen (N) is a limiting nutrient for plant growth in many forest ecosystems (Aber *et al*. 1989) and N supply is expected to limit the ecosystem-level carbon uptake and storage in many systems (Rastetter *et al*. 1997; Luo *et al*. 2006; Reich *et al*. 2006). Soil N availability is influenced by many transformation processes, such as N mineralization, nitrification, immobilization, volatilization, and denitrification, the first two being likely most important in terrestrial ecosystems. In N mineralization the organic N is transformed into inorganic N, whereas in nitrification NH_4^+ is oxidized into NO_3^-. The pool of ammonium and nitrate in terrestrial ecosystems is small, but there is a large annual flux through these pools (Schlesinger 1997).

As two fundamental processes of N cycling in terrestrial ecosystems, N mineralization and nitrification are still poorly understood in some forest ecosystems, including the broad-leaved forests and mixed forests (Nilsson *et al*. 1995). Moreover, information is generally lacking on the N mineralization and nitrification in subtropical forest ecosystems (Maithani *et al*. 1998; Upadhaya *et al*. 2005) and the data are extremely limited in subtropical China (e.g., Wang *et al*. 2007). There are reported short term soil N spatial variations (two months) in a mixed evergreen broadleaf forest in subtropical China (Wang *et al*. 2007), but the knowledge of a longer term mineralization dynamics in more diverse forest types is still unknown. In addition, although there is rich literature documenting the significant role of forest type on N cycling (Gower and Son 1992; Reich *et al*. 1997; Lovett and Rueth 1999), very few examined the effect of forest type by differentiating the contribution of microclimatic (e.g., temperature and soil moisture) and biotic factors (e.g. biodiversity).

The subtropical forests in southwestern China have been subjected to fragmentation by deforestation and over-exploitation, which resulted from regional socio-economic developments (Chen 2000a). Study on the N transformation dynamics and their controlling factors in this region will not only provide insights into N cycling in these ecosystems, but also provide valuable information for natural ecosystem restoration and forest management at a local level. In this study, we investigated N mineralization and nitrification dynamics in three subtropical forest ecosystems, i.e., a mixed evergreen broadleaf forest, a pure Zhennan (*Phoebe zhennan* S. Lee et F. N. Wei) forest plantation, and a pure Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) forest plantation using the closed *in situ* core incubation method. The evergreen broadleaf forest and Zhennan forest plantation are under similar local climate conditions but differ in plant biodiversity; the Zhenan and Chinese fir forest plantation are similar in plant biodiversity but under different local climate conditions. We also measured the soil organic carbon (SOC) and total N content changes during the same period. Environmental factors such as soil water content, soil temperature and throughfall were also measured.

The objectives of this study were 1) to determine the seasonal N mineralization and nitrification patterns of the three different forest sites under subtropical climate conditions, and 2) to determine the major controlling factors on N mineralization and nitrification in this region. We expect that 1) the N mineralization and nitrification in the three forest sites have similar seasonal patterns; 2) the rates of N mineralization and nitrification are different among the three forest sites; and 3) biodiversity may play an important role in rates and variability of the N mineralization and nitrification at the local scale. Due to human disturbances and over-exploitation, it is not very easy to find the true replicates for all three forest types; this non-replicated situation may limit the generalization of our findings.

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MATERIALS AND METHODS

Site description

Three study sites representing three different forest sites were selected in Dujiangyan city, Sichuan province in southwestern China. This region has a typical subtropical monsoon climate with cool, dry winters from December to February and humid, warm summers from May to October (Wang *et al*. 2007). Because of the insulation from the surrounding high mountains, there is generally no extreme cold temperature in winter. The mean annual temperature is 15.2°C, and the mean temperature for January is 4.6°C. The mean annual precipitation (MAP) is 1200-1500 mm, most of which falls in the summer from June to September. In the summer, this region is dominated by topographically induced rain due to the influence of Pacific southeastern monsoon (Chen 2000a). Major soil types in this region are classified as mountain brown yellow soil (equivalent to a US Soil Taxonomy Ultisols) according to the Chinese Soil Classification System (Chen 2000b).

The evergreen broadleaf forest site (31°03′ N, 103°43′ E) is a naturally regenerated secondary forest near Dujiangyan city. The general slope aspect is south facing and the average slope is about 30°. The site is on the east slope of the east rim of Tibetan Plateau with an elevation between 755-805 meters above sea level (m a.s.l). The dominant canopy species include *Castanopsis fargesii* Franch (DBH 10-100 cm), *Cyclobalanopsis glauca* (Thunb.) Oerst. (DBH 20-100 cm), *Machilus pingii* Cheng ex Yang (DBH 10-150 cm) and *Symplocos laurina* (Retz.) Wall (DBH 5-90 cm). The dominant understory species include *Camellia oleifera* Obel., *Lindera pulcherrima* (Wall.) Benth, *Ilex szechwanensis* Loes., *Pittosporum sahnianum* Gowda, *Bambusa omeiensis* Chia et H. L. Fung and *Ardisia japonica* (Hornsted) Bl. The dominant species on the forest floor are ferns, including *Microlepia marginata* (Houtt.) C. Chr., and *Arachniodes rhomboidea* (Wall.) Ching. The Zhennan forest site is located in a suburban area of Dujiangyan city (30°44′ N, 103°27′ E). It is 765 m a.s.l and has a slope of about 30°. The dominant canopy species is *Phoebe zhennan* S. Lee et F. N. Wei. The forest floor contains very few plant species, which include shrub species such as *Kalopanax pictus* (Thunb.) Nakai and *Broussonetia papyrifera* (L.) L. Herit. ex Vent, and grass species such as *Coniogramme caudiformis* Chimg et Shing, *Achyranthes bidentata* Bl. and *Ophiopogon japonica* (L. f.) Ker-Gawl. The Chinese fir forest site is located inside the Longchi National Park (31°11′ N, 103°31′ E), 34 kilometers northwest of Dujiangyan city with an elevation of 1440 m a.s.l and a slope of about 10°. The MAP in Longchi area is 1600~2000 mm. The dominant canopy species is *Cunninghamia lanceolata* (Lamb.) Hook. The understory is covered with ferns, such as *Allantodia squamigera* (Mett.) Ching and *Pteris setuloso*-costnla Hayata, all year round except in winter. The Zhennan forest was planted in the early 1940s, and the Chinese fir forest in 1958. To assess the biodiversity at each site, the species composition surveys were conducted in a 100 m × 100 m plot for each site, in June 2001 for the evergreen broadleaf forest and in October 2005 for the Zhennan and Chinese fir forests. All the individuals were identified to genus or species level and abundance data were recorded based on life forms (e.g., trees, shrubs) (**Table 6**).

Sampling methods and chemical analysis

Net N mineralization and nitrification were measured with an *in situ* core incubation method (Raison *et al*. 1987). For the evergreen broadleaf forest site, the whole plot of 100 m × 100 m was divided into 378 5 m × 5 m subplots. Three soil samples were taken from each of the 25 randomly selected subplots out of 378 in August 2000 (75 sampling tubes in total for each sampling time). Each sample was taken by driving a PVC pipe of 5 cm in diameter and 15 cm in depth into the soil. After sampling from each plot, one PVC pipe of the same size was then inserted into the ground nearby (20-30 cm away) to start incubation. Three such pipes per plot were inserted for incubation. Each pipe was covered with a plastic film at the top to prevent rain water from entering, and with a 1 mm mesh net at the bottom to allow water, gas exchange, and soil micro-fauna to enter (Binkley and Hart 1989). All PVC pipes were removed in October 2000 and three new pipes were inserted adjacent to each of the three original sampling spots. This was repeated at a bi-monthly interval until the experiment ended in June 2001. Due to the relative remote locations of the Zhennan forest and the Chinese fir forest, three samples from five randomly selected plots instead of from 25 5 m × 5 m random plots were taken with the same procedure every two months from August 2000 to June 2001 (15 sampling tubes in total for each sampling time at each site).

Soil temperatures from two different layers (0-5 cm and 5-15 cm) were measured using a digital thermometer (JM222, Jinming Instruments,Tianjin, China) at each sampling subplot together with soil sampling. One soil sample from each of the two layers (0-5 cm and 5-15 cm) in each sampling subplot was taken in the field, and sealed in a zip-lock bag for gravimetric soil water content measurements. The gravimetric soil water content is defined by (wet soil weight – dry soil weight)/dry soil weight × 100% and was immediately determined by oven-drying a subsample of ~5 g at 105°C for 48 h in the laboratory (Gardner 1986). Precipitation data were not attained at the same time with N mineralization and nitrification *in situ* measurement in 2000, and were made up in 2001. Twelve rain gauges (11 for Zhennan forest) were put inside each forest stand for monitoring monthly throughfall from July 2001 to July 2002.

Soil samples from each PVC pipe were thoroughly mixed and air-dried in a nearby station owned by the local forestry bureau of Dujiangyan City. The samples were then ground to pass a No. 10 sieve (mesh size 2 mm) after small plant parts and stones were removed within a couple of weeks. Each soil sample was subsampled (~ 6 g) and extracted with 30 ml 1 M KCl solution for NH_4^+ and NO_3^- analyses following the standard procedure (Keeney and Nelson 1982). The extracts were analyzed with a Segmented Flow Analyzer (Segmented Flow Analyzer, the scalar SAN^{plus}, The Netherlands). The extraction of NH_4^+ and NO_3^- from air-dried soil samples is a common soil test procedure although air-drying may result in changes in NH_4^+ and NO_3^- after a long storage (Maynard and Kalra 1993). The soil samples were further ground to pass 0.15 mm-mesh sieve and were subsampled for Kjeldahl total N analysis following standard procedure (Bremner 1996). Soil organic carbon (SOC) content was measured by the H_2SO_4-$K_2Cr_2O_5$ method (Bao 2000).

Calculation and statistical analysis

The net N mineralization rate (R_M, mg N·kg^{-1}·d^{-1}) was calculated by:

$$R_M = (C_{m1} - C_{m0}) / T$$

where C_{m1} and C_{m0} (mg·N·kg^{-1}) represent the final and the initial values of NH_4-N and NO_3-N during each incubation period, respectively, and T represents the days of incubation. The nitrification rate (R_N, mg N·kg^{-1}·d^{-1}) was calculated by:

$$R_N = (C_{n1} - C_{n0}) / T$$

where C_{n1} and C_{n0} (mg·N·kg^{-1}) represent the final and the initial values of NO_3-N during each incubation period, respectively. R_M and R_N for each forest site and incubation period were tested for normality before analysis.

Data were log or square root transformed to meet the normal distribution requirement when necessary. Two-way ANOVA was used to test the effects of forest site and incubation period on SOC content, total N content, net N mineralization, and nitrification. If forest site × incubation period interaction effect was found significant, one-way ANOVA was used to compare net N mineralization rates and net nitrification rates between forest sites for each of the five incubation periods. Environmental factors such as soil water content and soil temperature were compared by three-way ANOVA with forest site, incubation periods and depth as independent variables. Means separation was done using the Student-Newman-Keuls (SNK) multiple range test. Differences in throughfall between forest sites were compared by one-way ANOVA. Due to the human and animal (e.g., monkey) disturbances at the sites, there were always several sampling tubes taken out for each sampling period and the interrupted locations were not consistent, of course. This results in an unbalanced matrix structure for different time/site combination, which make repeated-measure ANOVA impractical. Our regular two-way or three-way ANOVA (compared with repeated-measure ANOVA) are subject to Type I

Table 1 Physical and chemical characteristics of the soil (0~10 cm) across the study sites (Values in the parentheses are standard errors; n=15 for two pure forest plantation and n = 25 for evergreen broadleaved forest).

Site	Sampling time (Year month)	Soil Organic Carbon (%)	Total N (%)	C/N	pH*
Evergreen broadleaf forest	2000 8-10	------	0.23 (0.01) Aa	------	3.66
	2000 10-12	2.36 (0.14) Aa	0.22 (0.01) Aab	10.72 Aa	3.65
	2000 12-2001 2	2.13 (0.09) Aab	0.20 (0.01) Abc	10.65 Aa	------
	2001 2-4	------	0.20 (0.01) Abc	------	3.70
	2001 4-6	1.96 (0.08) Ab	0.18 (0.01) Ac	10.89 Aa	3.59
Phoebe zhennan forest	2000 8-10	------	0.16 (0.01) Ba	------	4.07
	2000 10-12	1.64 (0.22) Ba	0.18 (0.01) Ba	9.11 Aa	4.07
	2000 12-2001 2	1.47 (0.13) Ba	0.16 (0.01) Ba	9.19 Aa	------
	2001 2-4	------	0.16 (0.01) Ba	------	4.02
	2001 4-6	1.89 (0.30) Ba	0.19 (0.02) Ba	9.95 Aa	4.00
Chinese fir forest	2000 8-10	------	0.39 (0.02) Ca	------	4.36
	2000 10-12	4.51 (0.12) Ca	0.46 (0.01) Cc	9.80 Aa	4.38
	2000 12-2001 2	3.19 (0.15) Cb	0.31 (0.01) Cd	10.29 Aa	------
	2001 2-4	------	0.33 (0.01) Cb	------	4.11
	2001 4-6	4.31 (0.08) Ca	0.39 (0.02) Cab	11.05 Aa	4.17

Different capital letters indicate different means between three forest sites.
Different lowercase letters indicate different means between sampling periods within one forest site.
*Soil pH values were attained from another study at the same sites during similar period (Cai and Huang 2006). Soil pH was measured by KCl extraction (soil: KCl = 1:2.5, w/v).

error (easier to reject the null hypotheses). However, since most of the statistical results we reported here were either very significant (e.g., $p < 0.0001$) or non-significant (e.g., $p = 0.4$), we feel any adjustments, if all possible, won't actually change the results and conclusions. Regression analysis between the rates of net N mineralization and nitrification, and soil water content, soil temperature and throughfall was conducted to determine the extent of contributions of different environmental factors to net N mineralization and nitrification. The N mineralization and nitrification rate were dependent variables. The soil water content, soil temperature and monthly precipitation were independent variables. The averaged N mineralization, nitrification rate, soil water content, soil temperature at each sampling time at each forest site was used in the regression analysis. All statistical analyses were performed using SAS (version 9.1).

RESULTS

Seasonal changes in SOC and total N content

Two-way ANOVA revealed significant forest site ($p < 0.0001$), incubation period ($p = 0.0006$) and incubation period × forest site ($p < 0.0001$) effects on SOC (**Table 1**). SNK test showed that the soil SOC content followed the order of Chinese fir forest site > evergreen broadleaf forest site > Zhennan forest site (**Table 1**). One-way ANOVA showed that, for the evergreen-broadleaved forest site, SOC content during October to December 2000 was significantly higher than during April to June 2001, but there was no seasonal change in SOC content for the Zhennan forest site (**Table 1**). For Chinese fir forest site, SOC content during October to December 2000 and during April to June 2001 was significantly higher than during February to April 2001, but did not differ between the first two periods (**Table 1**).

Two-way ANOVA revealed significant forest site, incubation period and incubation period × forest site effects on soil total N content ($p < 0.0001$ for all three cases, **Table 1**). SNK test showed that the soil total N content followed the order of Chinese fir forest site > evergreen broadleaf forest site > Zhennan forest site (**Table 1**). One-way ANOVA showed that, for the evergreen broadleaf forest site, soil total N content during August to October 2000 was significantly higher ($p < 0.05$) than that during December 2000 to February 2001, February to April 2001, and April to June 2001, respectively; soil total N content during October to December 2000 was significantly higher ($p < 0.05$) than that during February to April 2001. There was almost no seasonal change in soil total N content of the Zhennan forest site (**Table 1**). For the Chinese fir forest site, soil total N content experienced considerable changes, and was found to be the highest during the sampling period of October to December 2000 (**Table 1**). Soil C/N ratios did not vary much between forest sites or among sampling periods (**Table 1**).

Table 2 Two-way ANOVA results on net N mineralization rate with forest site and incubation period as independent variables.

Source	df	Mean Square	F Value	p
Forest site (FS)	2	0.0476	1.78	0.173
Incubation Period (IP)	4	0.6956	26.02	<0.0001
FS × IP	8	0.118906	4.45	<0.0001
Error	143	0.026732		

Seasonal changes in net N mineralization and nitrification

Two-way ANOVA revealed significant incubation period and incubation period × forest site effects on N mineralization ($p < 0.0001$ for both cases, **Table 2**) and insignificant forest site effect ($p = 0.173$, **Table 2**). SNK test showed that the net N mineralization rates during two subdivided winter incubation periods (December 2000 to February 2001 and February 2001 to April 2001) were significantly higher than the other three periods ($p < 0.05$). One-way ANOVA showed that, net N mineralization rates differed among the three forest sites in three out of the five incubation periods (**Fig. 1**). For the August to October 2000 and October to December 2000 periods, the net N mineralization rate on the evergreen broadleaf forest site was significantly higher than on the Zhennan forest site and there was no difference between the Chinese fir forest site and the evergreen broadleaf forest site or the Zhennan forest site. During December 2000 to February 2001, the Zhennan forest site showed the highest net N mineralization rate and there was no difference in N mineralization rate between the Chinese fir forest site and the evergreen broadleaf forest (**Fig. 1**).

Overall, the N mineralization on the three forest sites showed similar seasonal trends: net mineralization rates were the highest during December 2000 to February 2001 followed by the period of February 2001 to April 2001 (**Fig. 1**). During the other three incubation periods, N mineralization rates were below zero for all three forest sites except the evergreen broadleaf forest site during October to December 2000 period (**Fig. 1**).

Two-way ANOVA revealed a significant incubation period ($p < 0.0001$) and incubation period × forest site ($p = 0.0019$) effects on nitrification and marginal forest site effect ($p = 0.0831$, **Table 3**). SNK test showed that the nitrification rates during February 2001 to April 2001 were significantly higher than the other four periods ($p < 0.05$). One-way ANOVA showed that, for the period from August

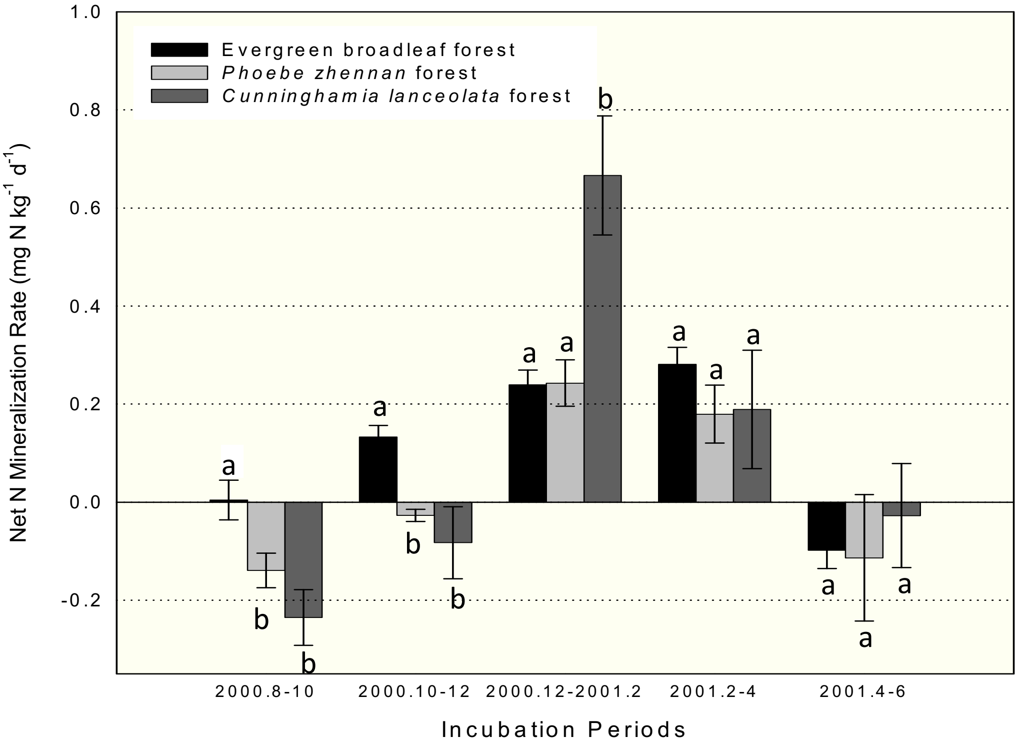

Fig. 1 Net N mineralization rates under three forest sites in five incubation periods. Different letters indicate significant different net N mineralization rate between the three forest sites at each incubation period (p<0.05). The error bars represent the standard errors of the net N mineralization rates.

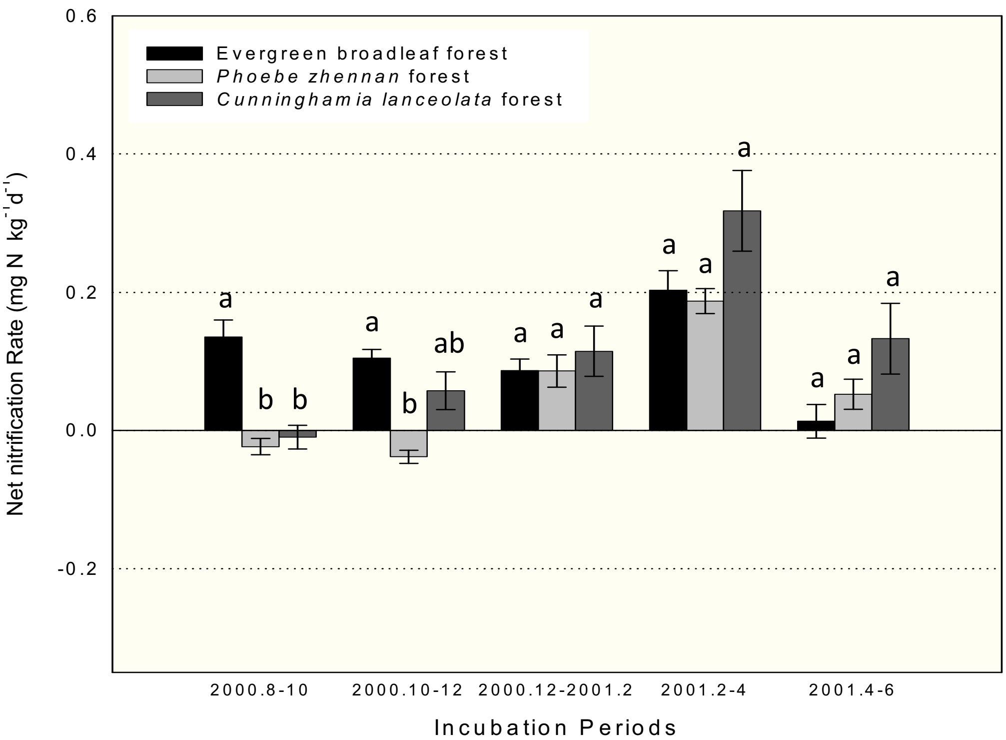

Fig. 2 Net nitrification rates under three forest sites in five incubation periods. Different letters indicate significant different net nitrification rate between the three forest sites at each incubation period (p<0.05). The error bars represent the standard errors of the net nitrification rates.

Table 3 Two-way ANOVA results on nitrification rate with forest site and incubation period as independent variables.

Source	df	Mean Square	F Value	p
Forest site (FS)	2	0.0242	2.54	0.0831
Incubation Period (IP)	4	0.0782	8.18	<0.0001
FS × IP	8	0.0313	3.27	0.0019
Error	143	0.00956		

to October 2000, the net nitrification rate on the evergreen broadleaf forest site was significantly higher than the other two sites (**Fig. 2**). From October to December 2000, the net nitrification rate on the evergreen broadleaf forest site was significantly higher than on the Zhennan forest site. The three forest sites showed no difference in nitrification rates of any of the other three time periods (**Fig. 2**).

Effects of environmental factors on N mineralization and nitrification

Three-way ANOVA revealed significant forest site ($p < 0.0001$), incubation period ($p < 0.0001$) and depth ($p = 0.0003$) effect on soil temperature (**Table 4**). There were also significant two-way and three-way interactions with exception of forest site and depth interaction (**Table 4**). Soil temperatures on the evergreen broadleaf forest site and the Zhennan forest site were significantly higher than on the Chinese fir forest site (12.8 and 12.8°C vs. 9.1°C; $p < 0.05$).

Three-way ANOVA revealed significant forest site and depth effect as well as significant interaction between them on soil water content ($p < 0.0001$; **Table 4, Fig. 3**). Soil water content differed significantly ($p < 0.05$) among the three forest sites, and followed the order of Chinese fir forest site (50.7%), evergreen broadleaf forest site (27.9%) and

Table 4 Three-way ANOVA results on soil temperature and soil water content (gravimetric) with forest site, incubation period and soil depth as independent variables.

	Soil Temperature			Soil Water Content (Gravimetric)		
	F	p	df	F	p	df
Forest site (FS)	3793.36	<0.0001	2	128.00	<0.0001	2
Incubation Period (IP)	12893.1	<0.0001	4	1.41	0.2306	4
Soil Depth (SD)	13.76	0.0003	1	148.76	<0.0001	1
FS × IP	93.78	<0.0001	8	0.57	0.7994	8
FS × SD	1.14	0.3204	2	21.35	<0.0001	2
IP × SD	25.69	<0.0001	4	1.26	0.2841	4
FS × IP × SD	6.75	<0.0001	8	2.19	0.0285	8
Error						

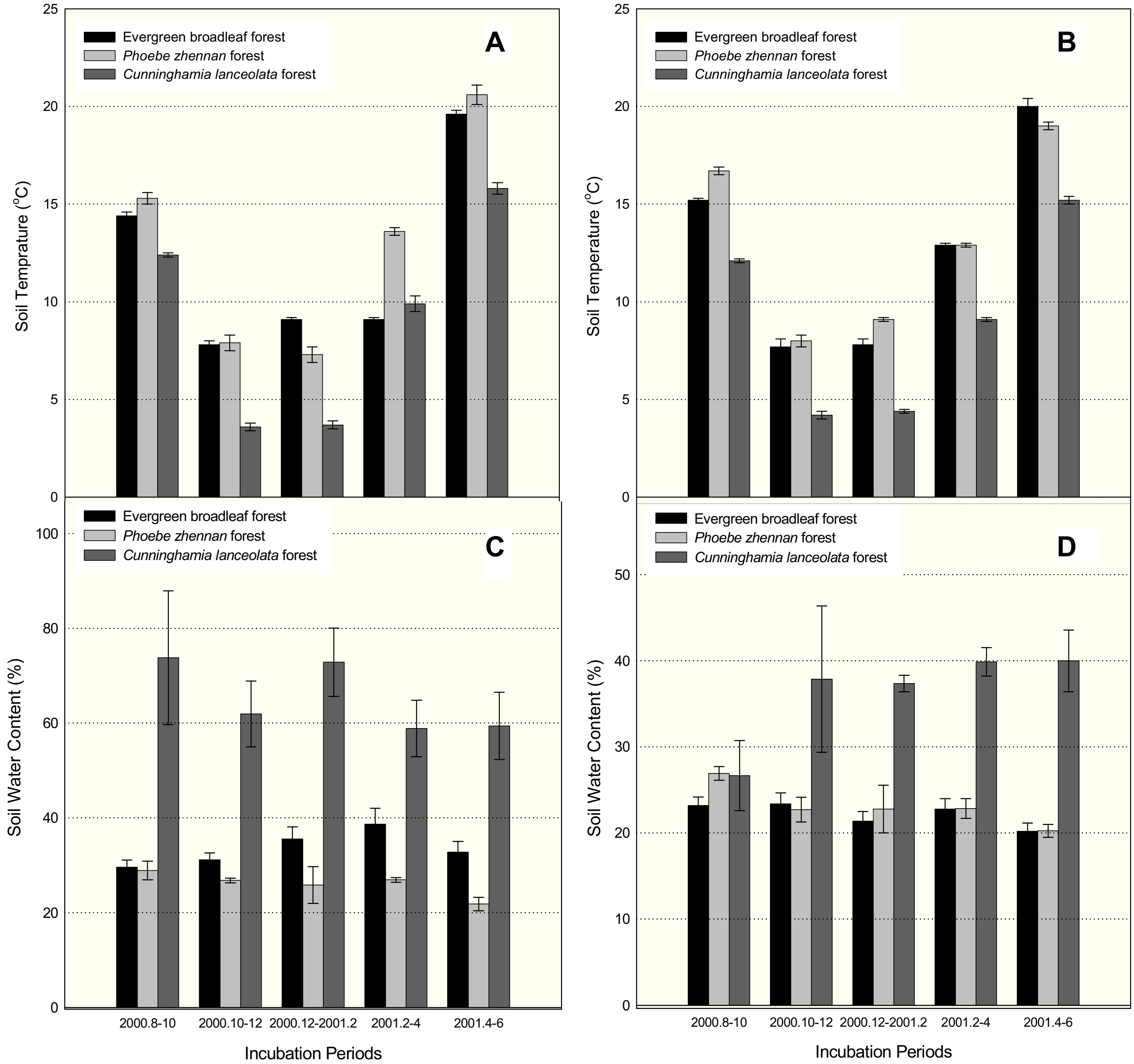

Fig. 3 Soil temperature of 0-5 cm (A) and 5-15 cm (B) and soil water content of 0-5 cm (C) and 5-15 cm (D) under three forest sites at five incubation periods. The error bars represent the standard errors of the soil temperatures or soil water contents.

Zhennan forest site (24.3%).

For both the evergreen broadleaf and the Zhennan forest sites, soil temperature and soil water content were not found to contribute significantly to either N mineralization (**Table 5**) or nitrification (**Table 5**). For the Chinese fir forest site, however, soil temperature contributed to net N mineralization and nitrification. For example, in the 0-5 cm soil layer, soil temperature explained 84.5% (p = 0.0272) of the total variance in the net N mineralization rates and 81.5% (p = 0.0358) in the net nitrification rates (**Table 5**).

Monthly throughfall did not differ among the three forest sites (F = 0.09, df = 2.28, p = 0.917), nor did it affect the N mineralization on any of the forest sites (**Table 7, Fig. 4**). However, throughfall significantly contributed to nitrification on the Zhennan forest site (**Table 7, Fig. 4**).

Biodiversity difference between three forest sites

Species richness differed markedly among the three forest sites. We surveyed species richness for three major func-

Table 5 Results of regression analysis (R^2) on net N mineralization rate (M) and net nitrification rate (N) with soil water content and soil temperature as independent variables for three forest sites and five incubation periods. The N mineralization and nitrification rate were dependent variables and the soil water content and soil temperature were independent variables. The averaged N mineralization, nitrification rate, soil water content and soil temperature of the whole site at each sampling period were used in the regression analysis.

		R^2 on M	R^2 on N
Soil Water Content			
Evergreen broadleaf forest	0-5 cm	0.394	0.402
	5-15 cm	0.101	0.281
Phoebe zhennan forest	0-5 cm	0.160	0.042
	5-15 cm	0.071	0.388
Cunninghamia lanceolata forest	0-5 cm	0.009	0.079
	5-15 cm	0.313	0.166
Soil Temperature			
Evergreen broadleaved forest	0-5 cm	0.005	0.182
	5-15 cm	0.029	0.144
Phoebe zhennan forest	0-5 cm	0.669	0.106
	5-15 cm	0.668	0.138
Cunninghamia lanceolata forest	0-5 cm	0.845 (-) *	0.815 (-)*
	5-15 cm	0.912 (-)*	0.198

Note: * < 0 .05.
"-" means negative influence.

Table 6 Species richness (defined by number of species per hectare) of different functional groups under three forest sites.

		Richness
Evergreen broadleaf forest	Dominant tree species	64
	Shrub species	102
	Herbaceous species	124
Phoebe zhennan forest	Dominant tree species	8
	Shrub species	14
	Herbaceous species	26
Cunninghamia lanceolata forest	Dominant tree species	1
	Shrub species	11
	Herbaceous species	15

Table 7 The contribution of precipitation to N mineralization and nitrification for each forest site. The N mineralization and nitrification rate were dependent variables and the monthly precipitation were independent variables. The averaged N mineralization and nitrification rate of the whole site at each sampling period were used in the regression analysis.

		R^2	p
Evergreen broadleaf forest	N mineralization	0.40	0.257
	Nitrification	0.58	0.077
Phoebe zhennan forest	N mineralization	0.44	0.337
	Nitrification	0.94 (-)	0.033
Cunninghamia lanceolata forest	N mineralization	0.62	0.211
	Nitrification	0.60	0.126

Note: "-" means negative influence.

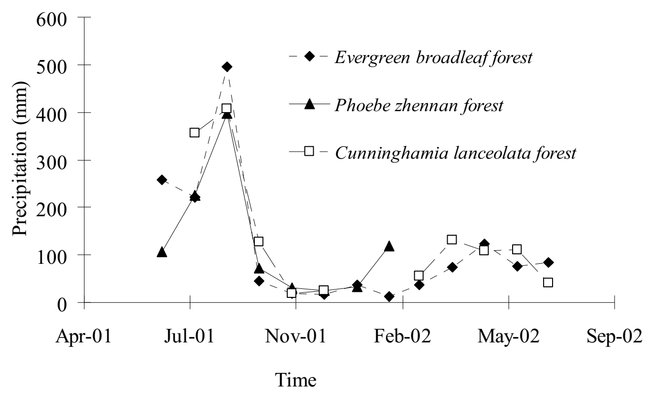

Fig. 4 Monthly canopy precipitation value (mm) under three forest sites from July 2001 to July 2002.

tional groups (i.e. dominant tree species, shrub species, and herbaceous species) on the three sites, and found that for all the three functional groups, the richness followed the order of the evergreen broadleaf forest site > the Chinese fir forest site > the Zhennan forest site (**Table 6**).

DISCUSSION

Dynamics of SOC, total N, net N mineralization and nitrification

The SOC and total soil N in all three forest sites are fairly stable over the course of the study period though variation exists between seasons (**Table 1**). Notably, the variations of SOC and total soil N between seasons at one particular location are not practically different though statistically significant (**Table 1**). The variations in total soil N in evergreen broadleaf forest are within the range from the same study site as reported in Wang *et al*. (2007). Both SOC and total N content are the highest for the Chinese fir forest site, followed by the evergreen broadleaf forest and the Zhennan forest plantation. However, there is no significant difference in soil C/N ratio among the three forest sites and soil C/N ratio does not change with seasonality (**Table 1**), indicating that the overall substrate quality for microbes on the three sites is similar and change little with seasonality.

The net N mineralization and nitrification rates found in this study are within the range of other studies with sites of similar climate patterns (e.g., a subtropical forest in India by Maithani *et al*. 1998). With the relatively low winter temperatures (December to February and February to April), it is intriguing that the highest rates for both net N mineralization and nitrification occur in winter for all three forest sites (**Figs. 1, 2**). Soil is very acidic for the three forest sites, which may inhibit nitrification process. Since there are no considerable changes in soil pH with seasonality (**Table 1**), change in soil pH is not the major driver for the observed variations in net N mineralization and nitrification. Closed-top PVC tube incubation method isolates incubated soil from plant uptake and excludes the N input of rainfall (Nadelhoffer and Aber 1985), so that net N mineralization rate measured in this study reflects the balance among ammonification, nitrification, denitrification, leaching and microbial immobilization. The fact that the highest net N mineralization and nitrification rates appear in winter times might result from decreased microbial immobilization or decreased denitrification in winter due to the lower temperatures (**Fig. 3A, 3B**). The decreased microbial immobilization in winter is supported by a recent experimental result of microbial N content at the same sites, which reveal higher microbial N content in summer time (Cai and Huang 2006). A further study is needed to test the denitrification scenario.

N is generally considered a limiting factor in boreal and temperate forests (Aber *et al*. 1989) and fewer studies on subtropical forest sites are reported (Maithani *et al*. 1998; Upadhaya *et al*. 2005). In this study, we find that only negligible net N mineralization and nitrification appear during growing season, which is in contrast to the findings of many other studies that mineralization rate was the highest in summer times (e.g., Knoepp and Swank 1998). Our results suggest that the microbial community on the three subtropical forest sites all suffer N limitation since microbes immobilize most of the mineralized N during the active growing season. However, the cause of such limitation remains unknown since neither litter C/N ratio nor soil C/N ratio in these forest ecosystems is very high. The soil C/N ratios are about 10 for all three sites (**Table 1**), which is typical in most ecosystems (Brady and Weil 1999). The litter C/N ratio in evergreen-broadleaf forest ranged from 27 to 44, and that of Chinese fir forest and the Zhennan forest are 59 and 58 respectively (Wang *et al*. unpublished data), which are within the range of other studies conducted in subtropical forests (e.g., Maithani *et al*. 1998). Such C/N ratios are much lower than those in Scots pine boreal forest (C/N ≈ 130, Staaf and Berg 1982), in temperate forests at Pacific

Northwest (C/N ≈ 70-100, Vitousek *et al*. 1982) and in young Hawaii tropical forests (C/N ≈ 110, Herbert and Fownes 1999), which are strong N limited ecosystems.

Although the overall net N mineralization and nitrification rates are similar for all three sites with the same seasonal trends (**Tables 2, 3** and **Figs. 1, 2**), the order of the rates in the three forest sites do vary seasonally (**Fig. 1**). Since the environmental factors such as soil temperature, soil moisture and precipitation change in similar manners in all three forest sites (**Fig. 3, 4**), the order of rates changes in net N mineralization and nitrification indicate strong vegetation effects, e.g. microbial community composition variations induced by different vegetation composition.

Controlling factors of net N mineralization and nitrification

Temperature and moisture are two major environmental factors affecting N mineralization and nitrification in soils (Sierra 1997). Soil water content can affect N mineralizetion and nitrification by changing water availability for microbial activity and controlling oxygen diffusion within the soil. In this study, soil water content does not significantly contribute to both net N mineralization and nitrification for all the three forest sites. The N mineralization and nitrification are not affected much by soil water content primarily because soil water content for all three forest sites are relatively high (30%-85%, **Fig. 3**) and may restrict microbial responses to soil moisture fluctuation (Chapin *et al*. 2002). Such high soil water content may also link to the N limitation in this region. In the arid environment, wetting-drying cycles promote the release of nutrients by increasing the turnover of microbial biomass and organic matter (Cui and Caldwell 1997). However, if water is not limiting, increased water availability (e.g. through increased precipitation) will exceed plant demand and decrease other resources such as N availability. For example, across a mesic to wet precipitation gradient in Hawaiian montane forest, Schuur and Matson (2001) found that increased MAP led to increased nutrient limitation and declined aboveground net primary productivity. The precipitation in this region is high (1200-1500 mm MAP) with additional foggy precipitation in the winter (personal observation), leading to a high soil water content (30%-85%, **Fig. 3**). Such high soil water content can lead to increased nutrient limitation due to N leaching process (Schuur and Matson 2001) or dissolved organic N loss (Perakis and Hedin 2002). The excessive humidity may also explain why throughfall is not found to contribute to net N mineralization for the three forest sites and has limited effect on nitrification (**Fig. 4**).

The effect of soil temperature on N mineralization and nitrification is through changing biochemical processes and oxygen consumption by microorganisms. N mineralization rate generally increases with temperature (Sierra 1997). In this study, however, we find a negative relationship between soil temperature and N mineralization rate (**Table 5, Figs. 1, 3A, 3B**), which suggests that N immobilization is more responsive to soil temperature than N mineralization. The effects of soil water content, soil temperature on N mineralization and nitrification may be under-estimated because of the relative short period with subsequent small sample sizes for regression analyses.

Net N mineralization and nitrification show similar seasonal trends for the three forest sites and the overall N mineralization and nitrification rates are similar among the three sites (**Figs. 1, 2**). These results support our hypothesis that long-term climate is the major controlling factor for net N mineralization and nitrification pattern at a regional scale. At the local scale, however, we think plant biodiversity plays an important role in net N mineralization and nitrifycation in this region. The argument is supported by three pieces of evidence. First, the species richness of the three functional groups is all much lower for the Zhennan forest site (pure forest plantation with little vegetation cover on the forest floor) than that for the evergreen broadleaf forest site (**Table 6**). As a result, with similar environmental variables such as soil temperature, throughfall and soil water content, the N mineralization and nitrification dynamics are quite different between the two forest sites, indicated by higher fluctuation and by higher contribution from environmental factors in Zhennan forest (**Figs. 1, 2**). Secondly, both the Zhennan and the Chinese fir forest sites are monocultures with similar species richness (**Table 6**). Although the soil temperature are significantly lower and soil water content are significantly higher in the Chinese fir forest site than those in the Zhennan forest site (**Fig. 3**), they show rather similar patterns in N mineralization and nitrification. Finally, the biodiversity effect on N mineralization at local scale is also supported by observations from other geographical regions, e.g. in south America temperate forest, Pérez *et al*. (1998) found that biodiversity related to forest ecosystem N mineralization dynamics. Pérez *et al*. (1998) suggest that, in the southern Chile temperate forests, two major factors might be related to higher biodiversity, i.e. fine-litter inputs with narrower C/N ratios and higher soil pH (broadleaf forest vs. conifer forest) contributed to higher N mineralization in higher biodiversity forest-broadleaf forest. In this study, soil pH values do not vary much among the forest sites and the evergreen broadleaf forest site is even lower in soil pH value (**Table 1**). However, the litter C/N ratio in evergreen broadleaf forest site is much lower than those of the Chinese fir and the Zhennan forest sites (27-44 vs. 58-59 in evergreen broadleaf forest and other two forest sites, Wang *et al*., unpublished data). Therefore, it is reasonable to conclude that the biodiversity induced N mineralization and nitrification dynamic differences between different forest sites in this region are mainly influenced by initial litter quality. This is supported by observations from three old re-growth subtropical forests in India (Maithani *et al*. 1998), in which the authors found that N mineralization in soil was significantly correlated with the quality of litter.

In summary, this study reports the seasonal pattern of N mineralization and nitrification in three subtropical forest sites in southwestern China. The results show that for three forest sites, rates of both net N mineralization and nitrification are the highest in the winter season (December 2000 to April 2001), and are negligible in spring and summer. The results also show that N is a potential limiting nutrient in these systems and high rainfall (high soil moisture) may be responsible for such limitation. The results indicate that regional climate may determine the patterns of N mineralization and nitrification at larger scale and biodiversity plays an important role at local scales. Under a similar climate condition, higher biodiversity could lead to higher and more stable N mineralization and nitrification, mainly through the effect of initial litter quality. Because of the rarity of these subtropical forest types in this region, mainly due to human disturbances and over-exploitation, we do not have true replicates for each forest type. This lack of replication places constraints on the generalization for these findings and readers should be cautious to apply these research findings to other subtropical forest ecosystems.

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