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REGULAR ARTICLE

Temporal patterns of net soil N mineralization and nitrification through secondary succession in the subtropical forests of eastern China

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Abstract Linking temporal trends of soil nitrogen (N) transformation with shifting patterns of plants and consequently changes of litter quality during succession is important for understanding developmental patterns of ecosystem function. However, the successional direction of soil N mineralization and nitrification in relation to species shifts in the subtropical regions remains little studied. In this study, successional patterns of net soil N mineralization and nitrification rates, litter-fall, forest floor litter, fine root and soil properties were quantified through a successional sequence in the subtropical forests of eastern China. Net N mineralization rate was 'U-shaped' through succession: highest in climax evergreen broad-leaved forest (CE: 1.6±0.2 mg-N kg^{-1} yr^{-1}) and secondary shrubs (SS: 1.4±0.2 mg-N kg^{-1} yr^{-1}), lowest in conifer and evergreen broad-leaved mixed forest (MF: 1.1±0.1 mg-N kg^{-1} yr^{-1}) and intermediate in conifer forest (CF: 1.2±0.1 mg-N kg^{-1} yr^{-1}) and sub-climax forest (SE: 1.2±0.2 mg-N kg^{-1} yr^{-1}). Soil nitrification increased with time (0.02±0.1, 0.2±0.1, 0.5±0.1, 0.2±0.1, and 0.6±0.1 mg-N kg^{-1} yr^{-1} in SS, CF, MF, SE and CE, respectively). Annual production of litter−fall increased through succession. Fine root stocks and total N concentration, soil total N, total carbon (C) and microbial biomass C also followed 'U−shaped' temporal trends in succession. Soil bulk density was highest in MF, lowest in CE, and intermediate in SS, CF and SE. Soil pH was significantly lowest in CE. Temporal patterns of soil N mineralization and nitrification were significant related to the growth of conifers (i.e. *Pinus massoniana*) and associated successional changes of litter-fall, forest floor, fine roots and soil properties. We concluded that, due to lower litter quality, the position of *Pinus massoniana* along the succession pathway played an important role in controlling temporal trends of soil N transformation.

Keywords Evergreen broad-leaved forest · Fine roots · Forest floor litter · Litter-fall · Soil incubation · Soil N availability · Soil properties

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Introduction

Changes of soil nitrogen (N) mineralization and nitrification through ecosystem succession have been received particular emphasis in recent decades (Vitousek and Reiners 1975; Lamb 1980; Robertson and Vitousek 1981; Pastor et al. 1987; Vitousek et al. 1989; Berendse 1990; Piccolo et al. 1994; Merilä et al. 2002; Bélanger et al. 2004; Pérez et al. 2004). However, discrepancies between studies remain unresolved. For instance, some results documented that potential N mineralization and nitrification generally increased through succession (Robertson and Vitousek 1981; Pastor et al. 1987; Berendse 1990; Knops and Tilman 2000), while other researchers found that soil N mineralization decreased during succession (Vitousek and Reiners 1975; Lamb 1980; Klingensmith and Van Cleve 1993; Van Cleve et al. 1993; Compton et al. 1998; Merilä et al. 2002). This variability can probably be explained by the original condition of the sites where succession initiated (Robertson and Vitousek 1981; Vitousek et al. 1989). Generally, succession starts from new substrates that invariably result in low soil N mineralization and nitrification early in succession (Vitousek et al. 1989; Berendse 1990; Pérez et al. 2004), while succession derived from destructive disturbance increases soil N mineralization and nitrification in recently disturbed sites but is then followed by a decline during later stages of succession (Vitousek et al. 1989; Piccolo et al. 1994; Mo et al. 2003).

Soil N mineralization and nitrification is influenced by many factors, such as soil substrate quality, soil concentration of phenolic compounds (tannins), soil pH, and activity of microbes (Vitousek et al. 1989; Berendse 1990; Northup et al. 1998; Gilliam et al. 2001; Bélanger et al. 2004; Bonifacio et al. 2008; Yan et al. 2008b). Change in patterns of these factors through succession has significant impacts on the direction of soil N mineralization and nitrification. For example, during secondary succession, soil N mineralization and nitrification changes significantly in response to changes of soil organic matter (Berendse 1990; Northup et al. 1998; Bélanger et al. 2004), phenol accumulation (DeLuca et al. 2002), and soil chemical and biological properties (Lamb 1980; Gilliam et al. 2001; Pérez et al. 2004; Bautista-Cruz and Castillo, 2005; Gilliam et al. 2005). Since plant litter is important in influencing these factors, the effects of succession on soil N mineralization and nitrification are found to be closely related to changes in litter quality and quantity (Berendse 1990; Northup et al. 1998; Mo et al. 2003; Bélanger et al. 2004; Pérez et al. 2004; Yan et al. 2008a, b).

However, changes in soil N mineralization and nitrification that relate to the nature of litter may be complicated by the functional differences of plant species, and the ensuing organic matter input and decomposition. Species with higher quality litter (i.e. a relatively low C:N ratio) are favoured over those with lower quality litter in improving soil N mineralization (Berendse 1990; Van Cleve et al. 1993; Northup et al. 1998; Paschke et al. 2000; Gilliam et al. 2001; Mo et al. 2003; Wang et al. 2007a; Bonifacio et al. 2008; Smal and Olszewska 2008). The high production of litter-fall and the high decomposition of litter would result in a high soil N mineralization in later-seral communities, relative to early-seral communities (Yan et al. 2008a).

The influence of plants on soil N mineralization rates, particularly those broadleaved species and conifers that occur at different successional stages, has been demonstrated in many chronosequences (Vitousek and Reiners 1975; Klingensmith and Van Cleve 1993; Piccolo et al. 1994; Compton et al. 1998; Knops and Tilman 2000; Merilä et al. 2002; Pérez et al. 2004; Wang et al. 2007a; Smal and Olszewska 2008). Interestingly, the observed patterns of soil N mineralization between broadleaved forests and conifer forests that occur in different successional sequences have been well researched. For example, conifers, regardless of whether they dominate at early or later-stages of succession, result in a lower net soil N mineralization in conifer forests compared to broad-leaved forests (Vitousek and Reiners 1975; Klingensmith and Van Cleve 1993; Merilä et al. 2002; Pérez et al. 2004).

Also, depending on their position in the successional sequence, conifers play an important role in controlling temporal trends of soil N mineralization and nitrification (Klingensmith and Van Cleve 1993; Van Cleve et al. 1993; Compton et al. 1998; Merilä et al. 2002; Mo et al. 2003; Pérez et al. 2004; Bautista-Cruz and Castillo, 2005). In general, the assembly of plants and the shifting patterns of conifers along successional gradients differ among biomes. In the boreal region, deciduous species are replaced by conifers, whereas, in the temperate region, conifers are substituted by broadleaved deciduous at late successional stages. As a result, soil N mineralization increases in later seres within temperature forests (Compton et al. 1998; Pérez et al. 2004), and decrease in the later successional stages of boreal forests (Klingensmith and Van Cleve 1993; Van Cleve et al. 1993; Merilä et al. 2002), respectively. However, in the sub-tropical area, conifers usually occur at early seral stages, dominate at intermediate-stages, and are replaced by evergreen broadleaved species at later-stages of succession (Yan et al. 2006). The shifting pattern of conifers along the successional sequence and their influence on soil N mineralization and nitrification in the subtropical region is thus different from those in the boreal and temperate regions. Consequently, we hypothesize that soil N mineralization and nitrification may be higher in early- and late-stages than in intermediate-stage of succession in the subtropical area.

In the sub-tropical China, the typical vegetation types are evergreen broad-leaved forests (EBLF, hereafter). Despite of their formerly widespread geographical distribution, the majority of the monsoon EBLF now exists as secondary forests (Yan et al. 2007; Wang et al., 2007b). Given the differences of anthropogenic disturbances, the forests become a diverse mosaic, and are representative of different successional stages. The pioneer plant species are often the mixing of those resprouting evergreen broadleaved species and conifers (Wang et al., 2007b). When the secondary succession proceeds, the conifer species such as *Pinus massoniana* is increasingly dominant in the coniferous forest. Thereafter, this species is gradually replaced by evergreen broad-leaved trees such as *Schima superba* in the sub-climax forests, and by *Castanopsis fargesii* in the climax forests (Yan et al. 2006).

In order to shed light on temporal patterns of soil N mineralization and nitrification in the sub-tropical chronosequence, we quantified in situ soil N mineralization and nitrification rates in five successional forests in Tiantong National Forest Park, Eastern China. Our specific objectives were: (1) to test the hypothesis that soil N mineralization and nitrification is higher in early- and late-stages than in intermediate-stage of succession; (2) to explore relationships of net N mineralization and nitrification with soil, fine root and litter properties; and (3) to examine the effect of seasonality on net soil N mineralization and nitrification rates.

Materials and methods

Study area and research site description

This study was conducted in Tiantong National Forest Park (29°48′N, 121°47′E,200 m a.s.l) located in Zhejiang province, Eastern China. The area has a typical monsoon climate with a hot, humid summer and a drier cold winter. The annual mean temperature is 16.2°C, the warmest month is July with a mean temperature of 28.1°C, and the coldest is January with a mean temperature of 4.2°C. Average annual precipitation is 1374.7 mm concentrated from May-August. The soils of this area are mainly red and yellow with a pH value ranged from 4.4 to 5.1. The substrate of parental material is composed of mesozoic sediments and acidic intrusive rocks, including quartzite and granite. Soil texture is mainly medium to heavy loam (Yan et al. 2006; Yan et al. 2008b).

The mature forests around a Buddhist temple in the centre of the park are considered as climax monsoon EBLFs because this area has been protected from clear cutting. Outside of this area, virtually all vegetation is secondary, and evergreen broad-leaved forest, coniferous forest and secondary shrubs occur widely. Due to the differences of abandoned ages following repeated cutting, these forests are representative of different successional stages and form a successional chronosequence. Based on forest types and dominant tree species, the series can be divided into five sucessional stages (Table 1). Generally, the secondary shrubs are mostly derived from the cessation of repeat cutting, and thus represent early successional stage. As the succession proceeds, shrubs will be replaced by coniferous forest, then which will be substituted by coniferous and evergreen broad-leaved mixed forest. Thereafter, succession will proceed to the sub-climax of evergreen broad-leaved forest, and finally to the mature-climax of evergreen broad-leaved forest (see details in Table 1).

Experiment design

We chose *C. fargesii*-dominated forests (climax evergreen broad-leaved forests, CE, hereafter), *S. superba*-dominated forests (sub-climax evergreen broad-leaved forests, SE, hereafter), *S. superba* and *P. massoniana* co-dominated forests (coniferous and evergreen broad-leaved mixed forests, MF, hereafter),

Table 1 Description of successional forests, indicating position in the successional chronosequence, community characteristics, stands age, and major disturbance regimes in Tiantong National Forest Park, Eastern China

Successi-onal stages	Forest types	Canopyheight (m)	Tree species richness	Dominated tree species and its importance value	Stand age (yr) [c]	Stands scope area(ha)	Forest management history and disturbance regimes
SS	Secondary shrub	6	4 [a]	*Lithocarpus glaber* (31) [b] *Pinus massoniana* (26) *Schima superb* (21)	~15	50	Derived from natural regeneration after cessation of the repeated cutting. With the supply of natural gas for cooking and heating, the clearance has been significantly reduced in recent decade.
CF	Coniferous forest	13	5	*Pinus massoniana* (52) *Schima superba* (35)	~25	6	Firewood extraction prior to abandonment. Attacked by pinewood nematode, and removed dead snags during the past decade.
MF	Coniferous and evergreen broad-leaved mixed forest	14	6	*Schima superba* (47) *Pinus massoniana* (40)	~35	8	Firewood extraction prior to abandonment. Attacked by pinewood nematode, and removed dead snags during the past decade.
SE	Sub-climax evergreen broad-leaved forest	15	7	*Schima superba* (66) *Castanopsis fargesii* (16)	~45	58	Snags and down deadwood harvesting. Nature disturbance regimes including typhoon and landslide.
CE	Climax evergreen broad-leaved forest	25	15	*Castanopsis fargesii* (72) *Castanopsis carlesii* (16)	~120	10	Protected from clear cutting. Canopy gap-phase dynamics presented. Typhoon is the major disturbance at regional scale with returning interval of 7–8 years.

[a] The dominant woody species are usually those multi-stems that regenerated vigorously from vegetative sprouts, [b] Importance value of tree species indicating its status in the specific successional forests. The 80% cumulative value was presented, [c] Years since abandonment, determined from the records of local forestry bureau.

P. massoniana dominated forests (coniferous forests, CF, hereafter) and secondary shrubs (SS, hereafter) as study stands, which represented five successional stages. The five vegetation types used for this study were located on the same position of the slope, had the same original vegetation in the history and the soils were developed from the same quartzitic parent material (Yan et al. 2006). We located four plots for each forest type. Study plots were separated by at least 50 m buffer zones and the size of plot was 20× 20 m.

In order to achieve our objectives, we split the study into two steps. The first step was to examine the in situ soil N mineralization and nitrification at five successional stages. Net soil N mineralization and nitrification rates in each plot were examined five times a year, with seasons defined according to plant phenology: spring, bud break (March, April and May; 95 days); early-summer, full leaf upgrowth (June, July; 50 days); middle-summer, full leaf mature (August, and September; 70 days); fall, abscission layer formation and leaf fall (October and November 60 days); and winter, dormant season (December, January and February; 90 days). In each of the five incubation periods, the field work in all plots was simultaneously completed within two days. The second step was to explore the patterns of litter-fall, forest floor, fine root and soil properties along succession, in order to relate it with soil N mineralization and nitrification.

Determination of soil N mineralization and nitrification

Soil N mineralization and nitrification rates were measured using in situ incubations. At the start of each incubation period, soil cores (positioned in pairs) were taken using PVC tubes (20 cm height and 7.5 cm in diameter) from five randomly chosen spots in each plot. The lower end of PVC pipes was sharpened to permit insertion with a minimum of compaction. The litter layer was removed before soil was taken. One of each pair of pipes (initial sample) was removed and returned to the laboratory in an icebox to determine initial soil ammonium (NH^+_4-N) and nitrate (NO^-_3-N) concentrations. The second pipe of each pair (incubated sample) was wrapped with low-density polyethene on the top and with gauze under the bottom that allowed gas movement, but prevented leaching, and then left in its original position from which it was taken. At the end of the each incubation period, the incubated samples were removed and the amount of mineral N accumulated was determined. Within each pipe, changes in inorganic-N content during the incubation period represent net N mineralized from organic sources (Rhoades and Coleman 1999).

All collected soil cores were kept cool until returned to the laboratory, stored in a refrigerator at 4°C until analyzed, usually within 36 hrs. In the laboratory, following removal of roots and stones, each soil core was well mixed by hand to form a homogenous sample and then passed through a ~6 mm sieve. One subsample of ~5 g were extracted in 20 mL of 2 *M* KCl for 1 hr and filtered through Whatman 42 filter paper. NH^+_4-N and NO^-_3-N were then analyzed with a Skalar flow-injection auto-analyser (Netherlands) using alkaline phenol and cadmium reduction techniques, respectively. To obtain oven-dry weight, one sub-sample (20 g) was placed in a 105°C oven for >12 hrs. Soil moisture was measured in a soil sample representing the incubated soil but was not the moisture inside the incubated core. The inorganic-N concentrations were expressed on a dry-weight basis.

Assuming no losses to leaching, plant uptake or gaseous N emissions, net N mineralization of a sample for the different incubation periods was obtained by subtracting the initial N (NH^+_4 and NO^-_3) concentration of the initial sample from that of the incubated sample. Soil net nitrification of a sample was obtained by subtracting the NO^-_3 concentration of the initial sample from that of the incubated sample. Net N mineralization and nitrification rates were obtained by dividing the incubation times from that of changes in inorganic-N (NH^+_4 and NO^-_3) concentrations during the incubation period and this was expressed as the net mineralization (or nitrification) per gram soil per day. Annual rates of N mineralization and nitrification were calculated by the sum of the values for all incubation periods.

Determinations of soil properties

Ten soil samples were taken with a metal corer from randomly chosen spots in each plot (0–20 cm soil layer). Approximately 4~5 soil cores per plot were placed in a 105°C oven for >48 hrs to estimate soil bulk density. The remaining 4~5 soil cores per plot were stored in a refrigerator at 4°C until analyzed, within 36 h. Then sub-samples (20 g) in each of the 100 fresh soil samples (5 forests × 4 plots × 5 cores) were used to examine soil microbial biomass Carbon (C). Soil microbial biomass C was estimated using the fumigation-extraction method (Vance et al. 1987). Firstly, a field-moist soil (equivalent to 20 g dry weight) with 40% of the field moisture capacity was fumigated with chloroform for overnight. After chloroform was removed, the sample was extracted with 0.5 mol L^{-1} K_2SO_4 (soil to extractant ratio of 1:5). The C concentrations of K_2SO_4-extracted solutions for the chloroform treated and untreated soils were measured using an automated TOC Analyzer (Shimazu, TOC-Vcph, Japan). Soil microbial biomass C was calculated by subtracting K_2SO_4-extracted C of untreated soils from that of the chloroform-treated soil, and calibrated using the extraction efficiency factor (Kc) of 0.38. Soil microbial biomass C was expressed on a wet-weight basis.

Thereafter, sub-samples in the remaining soil samples were air-dried for 30 days, and passed through a 0.5 mm sieve. The first subsamples of ~5 g in each of the 100 soil samples were analyzed for total N concentration using the flow-injection auto-analyzer, and these sub-samples were further ground to pass a sieve of 2 mm for total C concentration (oil bath-K_2CrO_7 titration method). The second subsamples were used to determine soil pH by using a Metterler-143 Toledo pH meter (1:2, H_2O).

Determination of litter-fall, forest floor and fine root properties

Three litter-fall traps of 0.5 m^2 were placed randomly at each of the plots. The distance between litter-fall traps was approximately 5 m. The traps were made of plastic net that allowed throughfall to percolate easily but retained litter particles. Usually, the traps were located at a height of 20 cm above ground. Litter-fall was collected on a monthly basis over a one year period. After air-drying, the litter was divided into leaf, twig, bark, flower, fruit and miscellaneous categories. The leaf litter was further subdivided into specific species. Any unidentified fine litter particles were added to a miscellaneous category. All litter components for each month were dried at 70°C constant for weighting, and then subsamples of approximate 50 g were ground and digested to analyze total N concentration, using flow-injection auto-analyzer (Skalar, Netherland).

Forest floor organic matter represented fine material at various stages of decomposition, including leaf litter and small branches (<2.5 cm in diameter). This organic matter came from both trees and understory vegetation in the plots. To account for the seasonal variations of litters, five samples were randomly collected in each of the plots every 3 months during a one year period. Forest floor organic matter was collected using a 0.4×0.4 m frame in the field, and returned to the laboratory in the plastic bags. All samples were oven dried at 70°C to constant weight, weighed and stored in a refrigerator (~4°C) to reduce decomposition until the fourth sampling was completed. Then samples from all four seasons were combined to give one composite sample. In total, there were 100 samples (5 forests × 4 plots ×5 spots). Subsamples of approximately 50 g were ground, and passed through a 6.0-mm sieve, and then digested to analyze total N concentration (Kjeldahl) using the flow-injection auto-analyzer. Total C concentration was determined using oil bath-K_2CrO_7 titration method.

The standing crop of fine roots (<2 mm in diameter) was determined by collecting five soil cores, 50 cm deep by 7.6 cm in diameter with a metal corer, in each plot. To reduce the seasonal variation of fine roots, the sampling also conducted every 3 months during a one year period. In total, there were 400 samples (5 forests × 4 plots ×5 cores ×4 times). In the field, each sample was placed in the plastic bags, refrigerated immediately upon return to the laboratory and processed within two days following harvest. Each core was soaked for at least 2 h, before being processed. Fine roots were sorted by tweezers, oven-dried at 70°C and weighed. Then subsamples were ground to determine total C and N concentrations (similar to forest floor).

Statistical analyses

Two-way ANOVA was used to determine whether there were significant successional effects on each response (N-mineralization, N-nitrification and soil moisture) over the five sample seasons. Successional stages and seasons were considered as fixed effects.

One-way ANOVA was used to determine the effects of successional stages on the net soil N mineralization and nitrification rates in different incubation periods and on the forest floor, fine root and soil properties. In these cases, the successional stage was included as fixed effect. If there was a significant effect for successional stage, least-squares mean separation with Tukey's correction was used to test for differences among successional stages. Also, Pearson correlation was used to relate soil N mineralization and nitrification rates with litter-fall, forest floor, fine roots and soil properties. All statistical tests were conducted using SPSS 11.5 and all statistical tests were considered significant at the $P<0.05$ level.

Results

Seasonality of net soil N mineralization and nitrification in relation to soil moisture

Net soil N mineralization and nitrification rates showed pronounced seasonality in the five successional forests (Table 2). Soil N mineralization and nitrification increased in the spring, early-summer and fall but decreased in the middle-summer and winter across the five forest types (Fig. 1). Net N mineralization was higher than soil nitrification in the spring, early-summer and fall (except for shrubs in the fall), and lower than soil nitrification in the middle-summer and winter (except for shrubs in winter and climax forest in middle-summer) (Fig. 2).

In the five forest types, soil moisture fluctuated significantly over the seasons (Table 2). It was lowest in middle-summer, highest in spring and/or in early-summer, and intermediate in fall and winter (Fig. 1). Considering all seasons, soil moisture in CE was significantly higher than SE, MF, CF and SS. With an exception of spring, soil moisture was significantly lowest in SS, and intermediate in CF, MF and SE in other seasons (Fig. 1).

Net soil N mineralization and nitrification through secondary succession

Net soil N mineralization and nitrification rates were significantly affected by the successional stage, seasonality and their interaction (Table 2). In the five incubation periods, soil N mineralization and nitrification exhibited different patterns through secondary succession (Fig. 2). As the succession proceeds from SS to CF, soil N mineralization decreased in the spring, summer and winter, but increased in the fall. Soil nitrification increased in the spring, early-summer and fall but decreased in the middle-summer and winter. During the successional stage of CF to the MF, soil N mineralization increased in the spring, early-summer and middle-summer and decreased in the fall and winter. Soil nitrification increased over all seasons. In the later two successional stages, soil N mineralization increased in spring, middle-summer and winter but decreased in early-summer and fall. Annual net N mineralization rate was 'U-shaped' through succession: highest in CE forest and SS, lowest in MF, and intermediate in CF and SE. Annual soil nitrification showed a increase trend through succession (Fig. 2).

Litter-fall, forest floor litters, fine roots and soil properties through secondary succession

Annual production of litter-fall gradually increased through succession, with the highest in CE and the lowest in SS. There was no significant difference in the N content of litter-fall among successional stages (Table 3). Forest floor properties varied across stages.

Table 2 Results of two-way ANOVAs for soil N mineralization, nitrification rates and soil moisture

Factor	df	Soil N mineralization rate		Soil nitrification rate		Soil moisture	
		F	p	F	p	F	p
Season	4	1557.8	< 0.001	62.8	< 0.001	67.7	< 0.001
Successional stage	4	5.8	< 0.001	34.2	< 0.001	23.4	< 0.001
Season-successional stage	16	72.5	< 0.001	8.4	< 0.001	11.6	< 0.001

The *F*-values and p-values are presented for effects of season, succession stages, and season-succession stages. Error df is 75 for soil N mineralization and nitrification rates, and soil moisture.

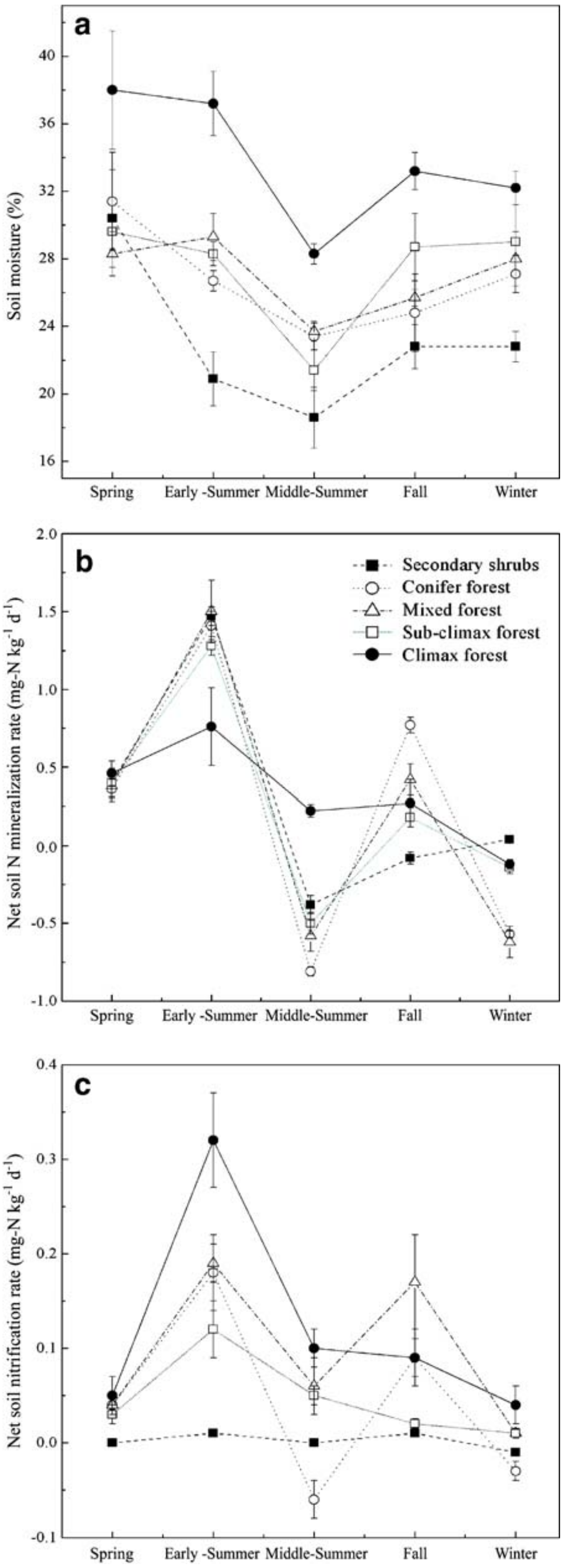

Fig. 1 Seasonal dynamics of soil N mineralization and nitrification rates and soil moisture among five successional forest types in Tiantong National Forest Park, Eastern China. Error bars show standard errors (n=4)

The stocks and the C mass were highest in SS, intermediate in CF and lowest in MF, SE and CE. The N concentration was lowest, and the C:N ratio was highest in SS than in other successional forests (Table 3).

Fine root stocks and N concentration showed a ‘U-shaped’ temporal trend through succession. The stocks of fine root were highest in SS, lowest in CF and intermediate in MF, SE and CE. The N concentration was lowest in CF, intermediate in SS, MF and SE, and highest in CE. In contrast, there was no significant change in total C concentration between forests. The C:N ratio was highest in CF and lowest in CE (Table 3).

Soil bulk density was highest in MF, lowest in CE, and intermediate in SS, CF and SE. Soil pH was lowest in CE, compared with the other four stages. Soil total N, total C and microbial biomass C also followed ‘U-shaped’ temporal trends through succession and were highest in CE, intermediate in SE and SS and lowest in CF and MF. Conversely, CF and MF had the highest, and CE and SS had the lowest soil NH^+_4 and NO^-_3 concentrations. Soil C:N ratios were significantly greater in CF and SE than in SS, MF and CE (Table 3).

Relationship of annual soil N mineralization and nitrification rates to litter-fall, forest floor litters, fine roots and soil variables

Litter-fall production was positively correlated with annual soil N mineralization and nitrification rates (Table 4). The N concentration in litter-fall was found to be significantly correlated with soil N nitrification rate, but was not correlated with soil N mineralization rate (Table 4). There was no significant correlation between annual soil N mineralization and the properties of the forest floor. In contrast, annual soil nitrification was positively correlated with N concentration and negatively correlated with stocks, C mass and C:N ratio in forest floor (Table 4).

There were significantly negative relationships of soil bulk density and soil pH with soil N mineralization and nitrification. Fine root stocks and total N concen-

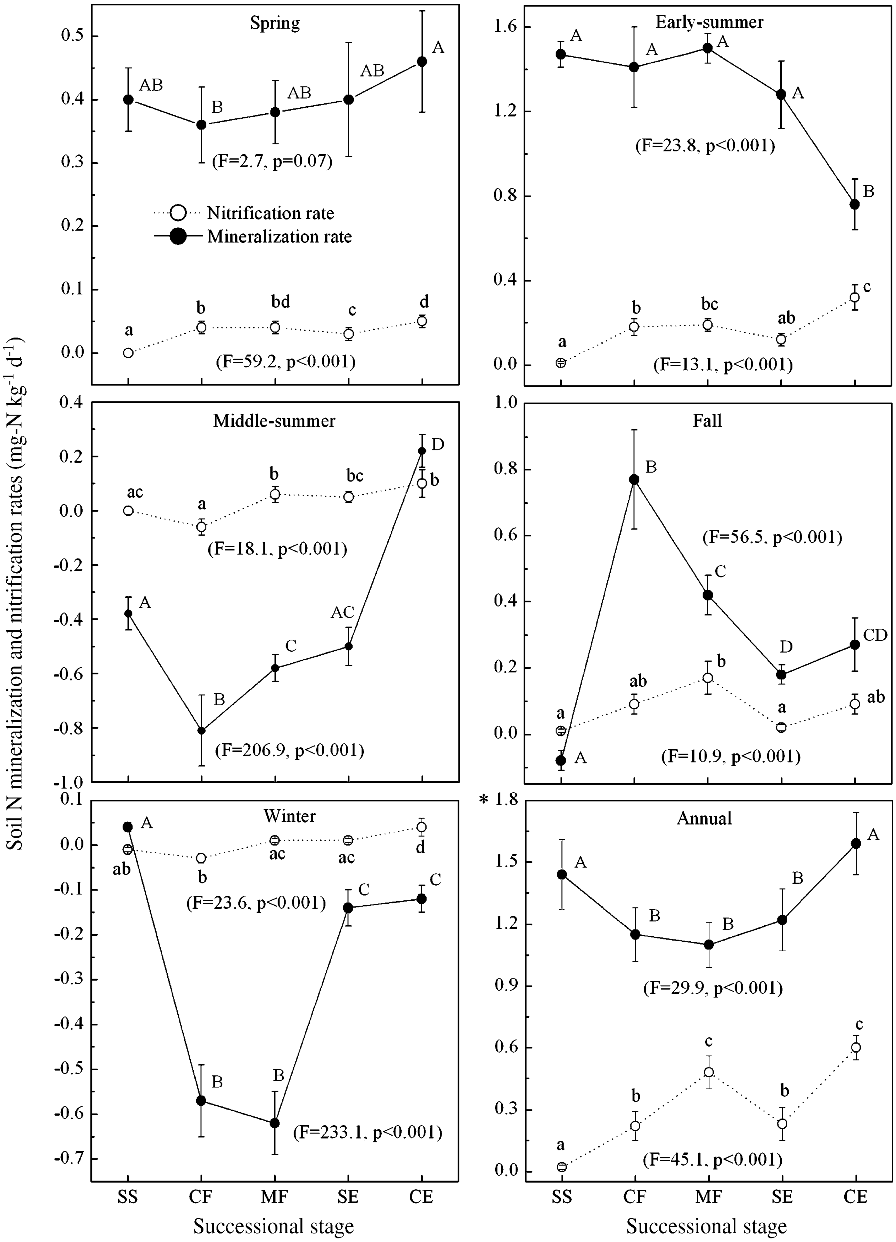

Fig. 2 Temporal patterns of net soil N mineralization and nitrification in different incubation periods along a successional gradient in Tiantong National Forest Park, Eastern China. Error bars show standard errors ($n=4$). Different letters in each line indicate significant differences between successional stages, and were tested using a one-way ANOVA with a *post-hoc* test of significance at Tukey's adjusted $p<0.05$. Data in parentheses show the F and p values, indicating the effects of succession on net soil N mineralization and nitrification. SS: secondary shrubs; CF: coniferous forest; MF: coniferous and evergreen broad-leaved mixed forest; SE: sub-climax evergreen broad-leaved forest; CE: climax evergreen broad-leaved forest; * near the Fig. 2 indicate that the unit of soil N mineralization and nitrification rates was mg-N kg^{-1} yr^{-1}

tration were positively and total C concentration and C:N ratio were negatively correlated with annual soil N mineralization. Surface soil total N, total C and microbial biomass C were positively correlated, but C:N ratio and soil NH^{+}_{4} and NO^{-}_{3} concentrations were negatively correlated with annual soil N mineralization rate. In contrast, total N concentration in fine root and soils were positively correlated, while the C:N ratios in fine roots were negatively correlated with annual soil nitrification rate (Table 4).

Discussion

Effects of seasonality on soil N mineralization and nitrification

Seasonality affects soil N transformations mainly through controlling temperature and water availability (Klingensmith and Van Cleve 1993; Piccolo et al. 1994). Low temperature and moisture reduce net soil N mineralization and nitrification (Pérez et al. 2004).

Table 3 Differences of litter-fall, forest floor litter, fine root and soil properties in five successional forests in Tiantong National Forest Park, Eastern China

Ecosystem properties		Secondary shrubs (SS)	Conifer forests (CF)	Mixed forests (MF)	Sub-climax forest (SE)	Climax forest (CE)	Successional stages effect (F=)
Litter-fall	Production ($g.m^{-2}$ yr^{-1})	637.8 (70.8) a	791.3 (18.8) b	839.3 (18.3) b	1012.8 (13.6)c	1323.5 (15.3)d	223.9***
	N content (%)	0.6(0.2)	0.6(0.1)	0.7(0.02)	0.8(0.01)	0.7(0.01)	0.3 (ns)
Forest floor litter	Mass($kg.m^{-2}$)	2.1 (0.4)a	1.7(0.2)ab	1.3(0.2)b	1.6(0.2)b	1.3(0.1)b	8.7**
	C mass (kg-$C.m^{-2}$)	5.3(1.2)a	5.0(0.5)a	3.2(0.4)b	4.0(0.7)ab	3.6(0.4)b	6.7**
	N content (%)	1.0(0.1)a	1.9(0.2)b	1.7(0.5)b	1.5(0.1)ab	1.7(0.2)b	8.4**
	C:N ratio	25.1(2.8)a	15.8(1.1)b	15.2(4.6)b	17.1(1.1)b	16.6(0.4)b	10.5***
Fine roots	Stocks ($t.hm^{-2}$)	5.7(0.2)a	0.7(0.1)b	1.6(0.2)c	2.7(0.1)d	3.2(0.2)e	458.4***
	N content (%)	0.8(0.1)a	0.6(0.1)b	1.0(0.1)c	1.1(0.2)c	1.4(0.2)d	136.5***
	C content (%)	39.4(1.0)ab	41.8(2.6)a	40.4(1.2)ab	39.5(3.8)ab	38.2(1.9)b	4.4*
	C:N ratio	49.7(1.9)a	74.0(8.2)b	40.9(2.6)c	37.2(1.3)c	28.3(1.2)d	75.6***
Soil	Bulk Density ($g.cm^{-3}$)	1.3(0.04)a	1.3(0.05)ab	1.4(0.06)b	1.3(0.04)a	1.1(0.06)c	54.7***
	pH value (2:1 H_2O)	4.2(0.08)a	4.2(0.05)a	4.3(0.1)a	4.2(0.05)a	3.8(0.07)b	29.9***
	N content (%)	0.3(0.05)a	0.2(0.03)b	0.2(0.03)b	0.3(0.04)a	0.5(0.07)c	287.8***
	NH^{+}_{4} concentration (g-$N.kg^{-1}$)	25.5(1.7)a	29.8(1.2)b	30.5(1.3)b	24.9(0.7)a	14.2(0.2)c	171.8***
	NO^{-}_{3} concentration (g-$N.kg^{-1}$)	0.4(0.1)a	2.9(0.3)b	2.4(0.6)b	0.8(0.2)a	1.1(0.2)a	41.4***
	C content (%)	2.8(0.2)ac	3.4(0.3)ab	2.0(0.1)c	4.5(0.6)d	5.6(0.7)e	41.8***
	C:N ratio	10.8(0.6)a	16.3(2.1)b	10.4(1.0)a	17.1(1.4)b	10.7(1.2)a	24.3***
	Microbial biomass C (mg-$C.kg^{-1}$)	258.2(5.2)a	220.5(10.6)b	211.6(8.1)b	271.9(13.8)ac	295.7(16.7)c	36.9***

* $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns, not significant,Effect of successional stages is presented as p values of one-way ANOVAs. Different letters in each row indicate significant differences (adjusted $p<0.05$). Data are means ± SE ($n=4$)

Table 4 Pearson correlation coefficients for the relationships between soil N mineralization and nitrification rates and litter-fall, forest floor litter, fine roots and surface soil properties in Tiantong National Forest Park, Eastern China

Ecosystem properties		Soil N mineralization rate	Soil nitrification rate
Litterfall	Production	**0.46***	**075****
	N content	0.33	**0.76****
Forest floor litter	Mass	0.12	**−0.74****
	N concentration	−0.31	**0.51***
	C mass	0.08	**−0.64****
	C:N ratio	0.33	**−0.61****
Fine roots	Stocks	**0.66****	−0.41
	N concentration	**0.5***	**0.67****
	C concentration	**−0.66****	−0.29
	C:N ratio	**−0.46***	**−0.53***
Surface soil	Bulk Density	**−0.88*****	**−0.46***
	pH value (2:1 H_2O)	**−0.73****	**−0.51***
	N concentration	**0.82*****	**0.53***
	NH_4^+ concentration	**−0.86*****	−0.44
	NO_3^- concentration	**−0.61****	0.25
	C concentration	**0.55***	0.33
	C:N ratio	**−0.45***	−0.34
	Microbial biomass C concentration	**0.76****	0.13

Note: All the significant correlations indicated in bold.

* $p<0.05$; ** $p<0.01$; *** $p<0.001$. $n=20$

The present study showed that net soil N mineralization and nitrification significantly increased from spring to early-summer and from middle-summer to fall, but decreased from early-summer to middle-summer and from fall to winter in all forests. These patterns were consistent with the seasonal dynamics of soil moisture (Fig 1), suggesting that seasonal dynamics of soil moisture played an important role in controlling net soil N mineralization and nitrification. In our study region, there is a very moist and hot season (Mei-Yu rain) from May to June (i.e. early-summer), which is characterized by the continuous heavy cloud and rain with high temperatures. Consequently, soil moisture and net soil N mineralization and nitrification were concurrently highest in this season. The pronounced decline of net soil N mineralization and nitrification from early-summer to middle-summer may be due to the decrease of soil moisture in July (Fig. 1). Since soil moisture did not decease significantly from fall to winter, the reduced net soil N mineralization and nitrification in this season may due to the decline of the temperature.

Direction of soil N mineralization and nitrification through secondary succession

Conifer species play a significant role in controlling the direction of soil N mineralization and nitrification through succession. In our studied sequence, conifer species (*P. massoniana*) primarily occurred in early-stages, dominated in middle-stages, and disappeared in late-stages of succession. Since patterns of conifer establishment through succession in sub-tropical forests differ from those in the boreal and temperate regions, we thus hypothesize that soil N mineralization and nitrification may increase early in succession, decrease in intermediate stage of succession and then increase again in climax forests (late-successional stages).

The results showed that the two processes exhibited contrasting patterns, however, with net N mineralization displaying a 'U-shaped' pattern with maxima in early and late stages, and with nitrification generally increasing with time. The direction of soil N mineralization along succession was consistent with the results reported by Vitousek and Reiners (1975) but did not support the generality that soil N mineralization either decreases (Klingensmith and Van Cleve 1993) or increases (Robertson and Vitousek 1981; Berendse 1990; Pérez et al. 2004) during succession. Our results for soil nitrification supported the finding that nitrate production may increase during succession (Lamb 1980; Robertson and Vitousek 1981; Pastor et al. 1987; Pérez et al. 2004), but failed to support the suggestion that nitrification declined during secondary succession (Van Cleve et al. 1993;

Compton et al. 1998) and the occurrence of an inhibition of nitrification in climax ecosystems (Lamb 1980; Robertson and Vitousek 1981).

The rates of soil N mineralization and nitrification are largely dependent on the soil substrate chemistry, soil concentrations of phenolic compounds (tannins), soil pH, soil C and N attributes, and the activity of microbes and mycorhiza (Vitousek et al. 1989; Berendse 1990; Northup et al. 1998; Gilliam et al. 2001; DeLuca et al. 2002; Bélanger et al. 2004; Bonifacio et al. 2008; Yan et al. 2008b). Changes in patterns of these factors induced by succession may be complicated by differences of plant species composition and the resulting organic matter input and decomposition (Yan et al. 2008a). In this study, the successional changes of litter-fall production and (or) N concentration, forest floor N concentration, fine root stocks and N concentration, and soil total N, total C and soil microbial biomass C concentrations were correlated with soil N mineralization and nitrification (see Table 3 and Table 4). In contrast, the successional patters of C concentration (or mass) and C:N ratios in fine root, forest floor and soil, soil bulk density and soil pH showed reverse patterns with net soil N mineralization and nitrification (see Table 3 and Table 4). This suggested that litter-fall, forest floor, fine root and soil properties were important controlling mechanisms of differences in N mineralization and nitrification in forest succession.

Shifts in plant species composition and resulting litter-fall, forest floor litter, fine roots quality and soil properties are the most probable explanation for the patterns of soil N mineralization and nitrification in secondary succession. Our results showed that secondary shrubs had high levels of soil N mineralization, which was consistent with other observations that the early successional stage generally involved a rapid increase in N availability shortly after disturbance (Vitousek et al. 1989; Mo et al. 2003). The higher soil N mineralization and the lower soil nitrification in SS could be attributed to following reasons. Firstly, due to the lower vegetation, increasing light reaches the forest floor and improves soil temperature, causing increased soil microbial activity. For example, in the spring incubation period of 2003, the average soil temperature in SS was 15.4°C, and in CF, MF, SE and CE was 11.2°C, 13.6°C, 13.7°C, and 15.1°C, respectively (Yan, unpublished data). Secondly, the relatively high soil N, $NH^+{}_4$ and microbial biomass C concentrations and relatively lower soil C:N ratios may elevate soil N mineralization (see Table 3). Finally, compared with CF and MF, the higher N concentrations in litter-fall and fine roots and the maxima of forest floor mass and fine roots stocks in SS may result in higher soil N mineralization rates.

Changes in N availability during secondary succession could continue after the initial pulse of increased availability disappeared (Vitousek et al. 1989). Generally, this change was caused by a shift of dominant trees during forest succession, especially the conifers, which can decrease soil N transformation through lower litter quality (Klingensmith and Van Cleve 1993; Piccolo et al. 1994; Compton et al. 1998; Merilä et al. 2002; Pérez et al. 2004; Bautista-Cruz and del Castillo 2005; Wang et al. 2007a; Bonifacio et al. 2008; Smal and Olszewska 2008).

In our studied region, following more than 25 years of secondary succession, *P. massoniana,* as a conifer species, almost became a monospecific dominant in the conifers forests, and then co-dominated with *S. superb* in the conifer and mixed forests. In these forests, due to the relatively lower quality of fine roots, litter and soils (i.e. lower N contents and higher C:N ratio, Table 3), and slower litter and fine root decomposition of *P. massoniana* (Yan et al. 2006; Yan et al. unpublished data), the forest floor litter accumulated to immobilize the majority of soil N (Vitousek et al. 1989), thus leading to the decrease in soil N mineralization. In addition, since conifer litter is a complex of tannins and phenols, it is less accessible for soil microbial decomposition than the new litter and labile root exudates (Northup et al. 1998; Gilliam et al. 2001; DeLuca et al. 2002; Bélanger et al. 2004), which would significantly reduce soil N mineralization. The rising of soil nitrification may mainly result from the forest floor accumulation that can protect inorganic N from soil erosion (i.e. higher forest floor mass, Table 3).

Improvements in soil N mineralization and nitrification in sub-climax forest and climax forest may reflect enhanced N and soil fertility (Vitousek and Reiners 1975; Vitousek et al. 1989; Lamb 1980). This could be true in the present study because litter-fall production and N concentrations among litter-fall, fine roots and soils were highest in CE, compared with those in early— and middle-successional forests (Table 3).

Conclusion

Due to lower litter quality, the differing position of conifers within the successional sequence of subtropical forests plays an important role in controlling temporal trends of soil N transformation. In the subtropical China, *P. massoniana* (conifer) occurs in early-stage, dominates in middle-stage, and disappears at the late-stage of succession. This pattern through succession differs from that of conifers in boreal and temperate regions. As a result, net soil N mineralization displays a ‘U-shaped’ temporal trend with maxima in early and late stages and with a minimum in intermediate stages of succession. Soil nitrification generally increases with time. Temporal patterns of soil N mineralization and nitrification are significantly related to shifts of conifer and ensuing successional changes of litter-fall, forest floor, fine roots and soil properties. Accordingly, in this special sequence, temporal patterns of soil N mineralization and nitrification differ from those chronosequences in boreal and temperate regions.

The mechanisms that underlie these patterns, such as concentration of phenolic compounds (tannins), structure and composition of microbe community and specific mycorhiza were not examined in the present study. Thus, there is a critical need for integrating these mechanisms in further research to examine soil N transformation.

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