Low N use Efficiency in A Long-Term N-Fertilized *Cunninghamia Lanceolata* Plantation Ecosystem in Subtropical China: A 14-Year Case Study

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Abstract

Background and aims

Forests host among the most important N pools of all terrestrial ecosystems. Influences of N application on forest N cycle have received increasing concern, which is particularly problematic given the increasing atmospheric N deposition in recent decades. However, accurate assessments of N storage and recovery rates in forest ecosystems remain elusive. We selected Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) plantation ecosystem to explore how long-term N fertilization affected the N storage and recovery rate.

Methods

Plots in the field have been fertilized continuously for 14 years (2004–2017) with urea at rates of 0 (N0, control), 60 (N60, low-N), 120 (N120, medium-N) and 240 (N240, high-N) kg N hm⁻²a⁻¹. Data collected in the field include N content and biomass on various plant organs (i.e., leaves, branches, stems, roots, and bark), understorey layer and litter in the ecosystem as well as soil N content and density at different depths (0–20, 20–40 and 40–60 cm).

Key results

The total N storage of ecosystem in the N-fertilized treatments was 1.1–1.4 times higher than that in the unfertilized treatment after 14 years of N fertilization. About 12.36% of the total ecosystem N was stored in vegetation (plant, litter, and understorey layer) and 87.64% was stored in soil (0–60 cm). N storage varied among ecosystem components and plant organs; and the plant organs, litter, and soil had higher N storage than that in understorey layer. Significantly higher Chinese fir N uptake was found in the medium-N (1.2 times) and high-N (1.4 times) treatments than that in the control. The N recovery rate of understorey layer in the N-fertilized treatments was negative, and less than that in the control.

Conclusions

Application of long-term N fertilizer to this stand led to a low N recovery rate (averagely 11.39%) while high loss of N (averagely 91.86%) which indicate low N use efficiency in the Chinese fir plantation ecosystem.

Introduction

Nitrogen (N) is a fundamental component of proteins, and nucleic acids, and as such, is a key regulator of vegetation growth and ecosystem productivity (Nadelhoffer et al. 2008; Xu et al. 2020; Qubain et al. 2021). Among the disturbances that affect terrestrial ecosystems, N is particularly sensitive to anthropogenic activity, including N fertilization (Bouwman et al. 2002; Tian et al. 2006; Etzold et al. 2020; Verma and Sagar 2020). The excessive and inappropriate application of N fertilizers induced by human activity may influence the global N cycle and alter forest ecosystem structure and function. For instance, N fertilization may inhibit ecosystem productivity by breaking nutrient balance (Nadelhoffer et al. 2008; Tian et al. 2018; Li et al. 2019a,b), reduce ecosystem biodiversity by soil acidification and ammonium toxicity (Talhelm et al. 2013; Zhang et al. 2014; Midolo et al. 2019; Wu et al. 2021), and even decrease ecosystem stability by affecting dominant species abundance, plant function groups composition, and constituent populations stability (Stevens et al. 2010; Koerner et al. 2016; Wu et al. 2020; Zhou et al. 2020).

Forest was the largest N pool in terrestrial ecosystems, and N storage of which accounted for 35.86% of the total N storage in China (Xu et al. 2020). Dynamics changes in forest N pools derived by N application alter the global N cycle process (Fenn et al. 2020; Xu and He 2020; Zhang et al. 2020). “Closed” N cycles are formed in the soil-litter-plant continuum found in forest ecosystems, and sensitive to N deposition (Nadelhoffer et al. 2008; Liu and Wang 2021; Qubain et al. 2021). In a closed N cycle, annual plant N uptake per unit area is balanced with annual N return to litter and soil (Nadelhoffer et al. 2008). The mass balance of N in a forest ecosystem is generally characterized by the difference between N inputs, vegetation and soil N sinks and N leaching and gaseous loss (Lovett and Goodale 2011). Fertilizers (especially soil fertilizers) are first transported into the soil and then absorbed and used by plants. Many studies have demonstrated that most N is retained by litter and soil, and only a small fraction is taken up and retained by plants (Nordin et al. 2001; Gurmesa et al. 2016; Jiang et al. 2018; Wang et al. 2018; Li et al. 2019b). The N uptake capacity of soils and plants in response to N addition may be the main factor determining tree growth and mortality (Wallace et al. 2007; Lovett and Goodale 2011). N from the forest ecosystem pool is subject to either plant uptake or various loss pathways, but a small fluctuation in the forest N pool can dramatically influence global N cycles (Xu and He 2020). The N recovery rate (*RE*) is relatively easy to measure and thus is often used as an indicator of N use efficiency (Davis et al. 2003). The N use efficiency was highest when N deposition was 40–100 kg N hm⁻² a⁻¹ (Gu et al. 2015). While higher N addition rates resulted in lower N leaching and less mortality might be the inherent

susceptibility which is mainly regulated by the N uptake capacity of soils and plants (Wallace et al. 2007). Nevertheless, the calculating of N recovery rate in forests is rare.

From previously published studies, we derived the following general understanding of the duration of most N fertilization experiments in China's forest ecosystems have been conducted short timescales (less than 10 years) (Gurmesa et al. 2016; Tian et al. 2018; Li et al. 2019b). For instance, 1–10 years of N application lead to elevated leaching of soil exchangeable base cations, decreased plant growth (Högberg et al. 2006; Li et al. 2019a). Two years N experiment showed that N-induced species loss increased more quickly at high rate and low frequency of N application (Zhang et al. 2014). Reports are rare for long-term (≥10 years) N fertilization at different doses. In the case of 150 kg N hm⁻²yr⁻¹ rate N fertilizer for 15 years, high tree mortality was induced (Högberg et al. 2006). Moreover, high rates of N application in 1–10 years may not represent the influences of the low frequency of N application over longer periods. However, despite numerous reports on the effect of N fertilization on plant growth and nutrient cycles in forest ecosystems, less is known about how extremely high N fertilization in forests influences N uptake and N storage in a longer perspective.

Excessive N fertilization enhances the leaching of ecosystem nutrients (Wilson and Tiley 1998). N leaching loss ranged from 2–20%, with a higher rate in plots treated with a high dosage of N (Tian et al. 2018). A recent study indicated that the threshold rate of N application for N leaching in N-saturated subtropical forests was 26–36 kg N hm⁻²a⁻¹ (Yu et al. 2018). Tian et al. (2018) reported that an N application rate exceeding 80 kg N hm⁻²a⁻¹ can be regarded as an extremely high level of N in forests. A rate higher than 90 kg N hm⁻²a⁻¹ can lead to negative feedback with respect to soil N availability and transformation (Verma and Sagar 2020). Moreover, high levels of N inputs have been suggested to cause forest decline in some areas (Wilson and Tiley 1998). In most cases, two symptoms of damage to forests is nutrient deficiencies caused by accelerated plant growth, a loss of soil base cations and species loss by soil acidification (Wilson and Tiley 1998; Högberg et al. 2006; Zhang et al. 2018).

In China, forest cover almost 0.22 billion ha, which correspond to 22.96% of the total area of terrestrial ecosystems (FAO 2015). China's plant forests has accounts for 27% of global planted forest area (FAO 2015; Zhang et al. 2020), of which Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) (*C. lanceolata*) is an economically important species in China. *C. lanceolata* does not fix N₂ and depend upon combined or fixed forms of N, i.e., N fertilizers (Tian et al. 2018). The average rate of N deposition in China has increased by approximately 60% over the past three decades (Yu et al. 2019). Total annual N deposition of north China ranged 21.9–41.2 kg N hm⁻² in 2011–2018, and these rates were higher than the peak value (10.02 kg N hm⁻²a⁻¹) in the United States and Europe (Yu et al. 2019; Luo et al. 2020).

The rate of N application in this study is much higher than the atmospheric N deposition rate (33.2 kg N hm⁻²a⁻¹ on average) across this region (Xu et al. 2018). Our previous studies pointed out that the same site as that examined in the current study could be N-saturated (Wu et al. 2017). An extremely high dose of N fertilizers (i.e., 120 and 240 kg N hm⁻²a⁻¹) provides a unique opportunity for studying the effects of high N inputs on the forest ecosystems. Here, we accurately estimated the N storage in *C. lanceolata* plantation ecosystem, including the allocation of N storage in various organs of plant (i.e., leaf, branch, stem, and root) and ecosystem components (plants, understorey, litter, and soils) and N recovery rate. The plantation received N fertilizer at four rates, i.e., 0, 60, 120, and 240 kg N hm⁻²a⁻¹, over a 14-year period. We hypothesized that: 1) *C. lanceolata* N storage will increase with rate under long-term N fertilization, so does ecosystem N storage; 2) N recovery rate might decrease with N application rate.

Materials And Methods

Study site

The study site was located in Guanzhuang National Forest Farm (117°43'E, 26°30'N), Sanming City, Fujian Province in southeastern China, at an altitude of approximately 200m. This area has a mid-subtropical monsoon climate with abundant rainfall, characterized by an average annual temperature of 20.1±1.96°C, precipitation of 2777±40.2 mm a⁻¹ (80% of which falls during May-October, Fig. S1), and frost-free period 271d (climatology based on measurements over 14 years from 2004 to 2017).

C. lanceolata plantation forest was planted in 1992 in an area of 6 hm² at a density of 1660 individual trees per ha⁻¹. In December 2003, twelve plots (20 m×20 m each and with a 15 m×15 m central area) were randomly selected within the forest, with the minimum distance between plots was 10 m. The average tree height of the whole was 12 m and the mean diameter at breast height (DBH, 1.3 m from the ground) was 16.1 cm. A survey conducted at this time revealed that understorey layer was sparse, with coverage between 3% and 5%, and dominant understorey species included *Miscanthus floridulus*, *Dicranopteris olichotoma*, and *Pteridium aquilinum* var. *Latiusculum* (Wu et al. 2013; Shen et al. 2019). We also measured soil physical and chemical properties at that time. Soil is acidic (pH=4.67), with an organic carbon content of 18.39 g kg⁻¹ and a total N content of 0.79 g kg⁻¹, and was classified as an Acrisol (Fan et al. 2007; Shen et al. 2019; Wu et al. 2021).

N fertilizer treatment

N fertilizer treatment in the twelve selected plots was initiated in January 2004 and continued for 14 years, until 2017. The N fertilizer treatments consisted of four levels, 0, 60, 120, and 240 kg N hm⁻²a⁻¹, referred to as N0 (control), N60 (low-N), N120 (medium-N), and N240 (high-N), respectively. Each treatment was applied to three replicate plots. According to the N concentration of each treatment, urea was dissolved in 20 L of water, and the solution was sprayed with a backpack sprayer on the forest floor in each plot every month.

Sampling

Sampling was conducted on December 15, 2017. According to the results of a per-wood inspection of all Chinese firs in plots in the previous period (2004-2017), we selected a standard tree (normal growth, no pests or diseases, and few scars) in each plot for wood test, thus, a total of twelve standard trees were selected for biomass measurements. We determined the orientation of the selected trees with a compass. Before felling, we marked the orientation of the stem at breast height. The central cross-sectional differentiation quadrature method was used to intercept the stem disc (5 cm thick) at the midpoint of each zone segment (every 2 m of the whole tree calculate initial from the coarse root of the plant). After felling, all leaves, branches, bark, and stem segments were taken from various point on the trees in the field and weighed. The root system biomass was measured following total root excavation. Root samples were divided into coarse roots (>10 mm) and fine roots (<2 mm, 2-5 mm, and 5-10 mm) and were cleaned with deionized water and weighed. Then all subsamples, 5 kg of each fresh weight, were transported to the laboratory.

Forest litter was sampled using a 1 m×1 m frame (three random samples per plot); the samples were separated into an undecomposed layer (L layer) and a semidecomposed layer (F layer), and the layers were then mixed into composite samples. We distinguished these two layers as follows: in the L layer, the original shape of the leaves is maintained, there is no significant change in colour and there is no superficial evidence of decomposition; by contrast, the leaves of the F layer do not have a complete outline, most of the litter has been crushed, and the mesophyll has decomposed into debris.

Soil samples were collected from three soil layers (0-20, 20-40, and 40-60 cm) using an auger (2.5 cm inner diameter). Subsamples of the soil cores (six cores randomly per plot) collected from the same layers in each plot were mixed into one composite soil sample. Soil bulk density was estimated using the core (5 cm inner diameter) method: the soil sample was oven-dried (105°C for 48 h) and bulk density was estimated as the mass of the oven-dried soil divided by its volume.

In order to capture the understorey diversity when more seasonal vegetation was visible, we investigated the diversity of understorey layer On September 16, 2017. Three 5 m×5 m survey subplots were randomly set in each plot, and the name, height and crown width of species as well as the number small trees, lianas, herbs and shrubs taller than 5 cm in each plot were recorded. The biomass of understorey layer was determined by harvesting; small trees and shrubs were collected from 2 m×2 m sampling areas, and herbs were collected from 1 m×1 m sampling area. Whole understorey plant were excavated and divided into above- and belowground components. The aboveground components consisted of leaves, branches and stems in the small tree, and leaves and branches in the herb layer, and the belowground components consist of roots. The fresh weight were measured, and transported to the laboratory to determine the water content and to calculate the dry biomass and N content of the understorey layer.

The stem disc samples taken at breast height were air-dried in ventilated conditions after which they were sanded with sandpaper until the annual rings were visible. The discs were then cross-dated to the tree core samples at each sample point. After that, the tree-ring width was determined using LinTab 6 (TSAP-Win, Germany).

Chemical analysis

The water content (%) of all the subsamples was determined in the laboratory. The soil samples were sieved through a 2-mm mesh screen to remove stones and roots and the soil water content was determined by drying at 105°C to a constant mass. Fresh leaves were first washed with 10% dilute hydrochloric acid and then repeatedly washed with deionized water to completely remove dust and particulates adsorbed on the surface, then killed in an oven at 105°C for 2 h, and then dried at 60°C to a constant mass. The remaining samples were cleaned and then dried at 60°C to over-dry. The dry biomass of each organ of the tree was calculated per unit area based on the water content.

All the subsamples were crushed with a microplant crusher, passed through a 100-mesh sieve, and kept in a dry environment prior to measurement. The total N and total C contents of the samples were analysed by an elemental analyser (PerkinElmer Corporation, 2400II, USA).

Calculation

(1) Basal area increment

We calculated the basal area increment (BAI, cm²) using the following equation:

$$BAI = \pi (R_n^2 - R_{n-1}^2) \quad (1)$$

where n is the number of tree rings and R is the radius of the tree (cm).

1. Soil N storage

Soil N density (N_d , kg m⁻²) refers to the storage of N in the soil layer at a specific depth per unit area and was calculated in the following equations:

$$N_{di} = 0.1 \times T_{Ni} \times \gamma \times H_i \times (1 - \delta/100) \quad (2)$$

where 0.1 is the conversion factor, i represents the soil layer, cm; T_{Ni} represents the total soil N content, %; γ represents the soil bulk density, g cm⁻³; H represents the soil depth, cm; and δ indicates the percentage of gravel in soil with a diameter ≥ 2 mm, %.

Soil N storage (N_s , t hm⁻²) was calculated as:

$$N_s = \sum (N_{di} \times A) \quad (3)$$

where A represents area, hm⁻².

1. Plant N uptake and recovery rate

RE_N was calculated by the difference method developed by Davis et al. 2003 and is defined as:

$$U_N = \text{Biomass} \times N \% \quad (4)$$

$$RE_N = (U_N - U_0) / R_N \times 100\% \quad (5)$$

where N (%) is the N content of major ecosystem components; biomass (t hm⁻²) is the plant biomass of a plot in this study; U_N (kg N hm⁻²) is the total plant N uptake measured in above- plus belowground biomass in a plot that received N dose (R_N) at i.e., 0, 60, 120 or 240 kg N hm⁻²a⁻¹; and U_0 is the total plant N uptake in unfertilized N plots (N0).

Statistical analysis

We used two-way ANOVA to test the effects of N fertilizer treatments, tree components, and soil layers on biomass, N concentration, and N storage. One-way ANOVA with Dunnett's post-hoc test was used to test the effects of the N fertilizer treatments on plant biomass, N content, and N uptake, understorey species, soil N content, and N storage in the soil layers. All analyses were conducted using SPSS software (version 19.0; SPSS Inc., Chicago, IL, USA). Figures were created with SigmaPlot 13.0 software (Sysat software, USA). Results were considered as significant at $P < 0.05$.

Results

Variation in soil N storage

There were significant effects of N fertilization on the N content of all three soil layers, i.e., 0–20 cm ($P = 0.018$), 20–40 cm ($P = 0.048$) and 40–60 cm ($P = 0.003$) (Table 1, Fig. 1a). N fertilization increased the soil N content (Fig. 1a), especially in N120 (+ 51.49%), followed by N240 (+ 20.66%) and N60 (+ 19.24%). N fertilization also significantly promoted soil N storage ($F = 13.040$, $P = 0.002$), particularly in N120 (Fig. 1b). Total soil N storage (0–60 cm) showed a trend of increasing and then decreasing with increasing N application, in the order of N120 (9.7490 t N hm⁻²) > N240 (8.3633 t N hm⁻²) > N60 (7.8743 t N hm⁻²) > N0 (7.4820 t N hm⁻²) (Fig. 1b). There was a significant difference in all three layers between the four N treatments ($P = 0.024$, $P = 0.044$, $P = 0.014$; Table 1, Fig. 1b). The N240 treatment increased soil N storage in the 0–20 cm layer by 32.53% compared to that in the N0 treatment but did not have an effect in the 20–40 cm or 40–60 cm layers.

Table 1 Two-way ANOVA of soil density, N content, and N storage in three soil layers under different N treatments

Variables	F (P) value					
	Density (kg m ⁻²)		N content (g kg ⁻¹)		N storage (t hm ⁻²)	
Treatment	2.898	0.056	18.711	0.001	5.392	0.027
Layer	11.948	0.001	4.99	0.015	12.00	0.001
Treatment × Layer	0.877	0.526	0.603	0.725	0.877	0.527

Variation in DBH and BAI

There was no significant difference in DBH between N fertilizer treatments ($P>0.05$; Fig. 2a). We divided the DBH values into classes (4 cm interval) from 10 cm to 38 cm. Large amounts of N fertilizer significantly decreased the number of trees with a DBH between 10 and 14 cm but increased the number of trees with a DBH > 30 cm and significantly increased mortality, especially in the N120 treatment ($P\leq0.05$; Fig. 2b). There were no trees with a diameter less than 10 cm in the N240 treatment, and in the 10–14 cm DBH class, there was a significant difference between the numbers of tree in the N240 and N0 treatments ($P\leq0.05$). The rate of tree mortality was 2.98%, 2.55%, 8.76%, and 7.32% in N0, N60, N120, and N240, respectively, representing a significantly lower mortality in N0 and N60 than in N120 and N240. During the N treatment period (2004–2017), a significant difference in BAI and annual BAI between N treatments occurred only in 2013 ($P=0.032$) and 2014 ($P=0.031$) (Fig. S2).

Variation in vegetation N storage

The dry mass and N content are similar within each plant organ between N treatments (Table 2). N concentration of leaves and fine roots indicate higher sensitivity than other tree samples to N fertilization, approximately two to four times. We calculated plant N uptake based on the N content and dry mass. There was a significant difference in the total of plant N uptake in 2017 ($F=7.906$, $P=0.009$) and their increments from 2003 to 2017 ($F=60.257$, $P<0.001$) between N treatments (Fig. 3); plant N uptake in the N120 and N240 treatments increased by 27.61% and 39.16%, respectively, relative to that in N0 (Table 2 and Fig. 3). N uptake of plant organs (leaf, branch, stem, and bark) under N fertilizer increased by 11.7%, 11.15%, 37.45%, and 24.6%, respectively, compared with the control (Table 2 and Fig. 3).

Table 2
Dry mass and N content of Chinese fir plant organs under different N fertilizer treatments in 2017. Values are means ± SEs (n = 3).

Component	Dry mass (t hm ⁻²)				N content (g kg ⁻¹)			
	N0	N60	N120	N240	N0	N60	N120	N240
Plant								
Leaf	7.55(0.14)	7.36(0.13)	7.67(0.14)	7.82(0.14)	12.81(1.30)	13.31(1.03)	14.23(1.65)	14.99(1.07)
Branch	19.13(0.50)	18.46(0.47)	19.58(0.52)	20.10(0.51)	6.94(0.31)	7.11(0.43)	7.73(0.54)	7.97(1.18)
Stem	115.25(4.01)	109.71(3.67)	118.90(4.23)	122.79(4.12)	3.00(0.35)	3.48(0.40)	3.78(0.46)	4.86(0.49)
Bark	16.87(0.45)	19.48(0.24)	22.91(0.78)	18.55(0.36)	7.62(0.12)	7.59(0.23)	8.01(0.18)	8.18(0.07)
Coarse root	35.77(4.82)	34.17(2.41)	42.62(11.61)	33.60(3.37)	4.97(0.15)	5.29(0.21)	5.76(0.27)	6.41(0.59)
Fine root								
< 2 mm	0.57(0.07)	0.53(0.22)	0.32(0.10)	0.37(0.05)	13.87(0.48)	13.82(0.86)	14.55(1.54)	15.36(1.28)
2–5 mm	0.97(0.23)	0.86(0.28)	0.65(0.13)	0.62(0.22)	12.88(0.37)	12.65(0.48)	12.47(1.42)	13.48(0.73)
5–10 mm	0.79(0.16)	0.67(0.14)	0.66(0.35)	0.86(0.18)	11.98(0.43)	12.87(0.49)	13.66(1.24)	14.44(1.47)
Subtotal	196.87	191.24	213.31	204.71				

The diversity of the understorey layer decreased with increasing levels of N treatment (Fig. 4a), with species counts of 50, 33, 28, and 16 in N0, N60, N120, N240, respectively. Regarding the different functional groups, the numbers of small trees and lianas decreased sharply (approximately 4-fold) in N120 and N240 ($P\leq0.05$) compared to that in N0; this pattern was not observed for shrubs or herbs (Fig. 4a). Similarly, the total, aboveground, and belowground understorey layer biomasses in the N-fertilized treatments (N60, N120, N240) were significantly lower than those in N0 ($P\leq0.05$; Fig. 4b). A significant difference existed between N60 and N240 in the total and aboveground biomass (Fig. 4b). Understorey layer N content showed no difference (Fig. 4c) while N uptake significantly decreased with increasing N fertilization levels ($P\leq0.05$; Fig. 4d), and aboveground N uptake contribute an average of 64.69% of the total N uptake.

Significant differences existed in total litter biomass under the different N fertilizer treatments (Fig. 5 and Table 3). The total litter biomass in N60 (5.23 t hm⁻²) was higher than that in N0 (2.55 t hm⁻², i.e., 104.81% higher) ($P\leq0.05$; Fig. 5a and Table 3). The biomass of F-leaf and F-branch in

N60 was more than 1.5 and 4.5 times higher, respectively, than that in N0 (Fig. 5b). N fertilization increased litter N content (Fig. 5c), especially N120 treatment ($P=0.001$), compared to that in the control. The highest increases in N uptake were observed in N120 for the total litter and components (leaf and branch) (Fig. 5d and Table 3). Specially, N fertilization had significant effects on the N content on both leaf litter ($F = 15.577$, $P=0.001$) and branch litter ($F = 25.026$, $P=0.001$; Fig. 5c and Table 3). Leaves litter contributed more to total litter N uptake (up to 79.41%) than branch litter. The branch N uptake in N120 was more than 4.5 times higher than that in N0.

Table 3 Two-way ANOVA of biomass, N content, and N uptake under different N treatment and component in litter

Variables	F (P) value					
	Biomass (t m ⁻²)		N content (g kg ⁻¹)		N uptake (t hm ⁻²)	
Treatment	5.367	0.003	14.865	0.001	10.928	0.001
Component	39.727	0.001	32.267	0.001	66.213	0.001
Treatment × Component	0.505	0.681	0.903	0.461	0.590	0.625

Note: Component: the leaf and branch in litter; Treatment: N fertilizer treatment, as N0, N60, N120, and N240.

Variation in ecosystem N use efficiency

- Among the major components in the plantation ecosystems, only the litter and soil N content were differed significantly between the N treatments (Fig. 6). N fertilization promoted total ecosystem N storage (Table 4), with average N storage of 12.10% in vegetation and 87.9% in soil in the N fertilizer treatments and 13.46% in vegetation and 86.54% in soil in the control.
- The plant N recovery rate in N120 was much higher than that in N60 and N240. The N recovery rates in vegetation were 2.41%, 19.11%, and 12.67% in N60, N120, and N240, respectively; the N recovery rate of understorey layer in the N fertilizer treatments were below zero than in control (Table 5). In contrast, the N recovery rates of the plants and litter were all positive among N treatments, and the N recovery rate was highest in N120, i.e., 20.98% in plants and 3.00% in litter. The N storage of vegetation was 14.44, 229.27, and 304.07 kg N hm⁻² higher in N60, N120, and N240, respectively, than in N0 (Table 6). When N storage in vegetation was compared with the total amount of N fertilizer from 2004 to 2017 (Table S7), the loss of N was estimated to be 825.56, 1450.73, and 3055.9 kg N hm⁻² in N60, N120, and N240, representing N loss percentages of 98.28%, 86.25%, and 90.95%, respectively. Together, our results suggested that long-term N fertilization might induce low ecosystem N use efficiency.

Table 4
N storage in Chinese fir plantation ecosystem (t N hm⁻²)

N fertilizer rate (kg N hm ⁻² a ⁻¹)	Chinese fir plant	Litter	Understorey vegetation	Soil	Total in ecosystem
N0	0.91 ± 0.03	0.0199 ± 0.0019	0.0752 ± 0.0068	6.47 ± 0.46	7.48 ± 0.26
N60	0.97 ± 0.04	0.0318 ± 0.0024	0.0219 ± 0.0050	7.87 ± 0.15	8.90 ± 0.10
N120	1.17 ± 0.10	0.0559 ± 0.0113	0.0167 ± 0.0017	9.75 ± 0.20	10.99 ± 0.15
N240	1.27 ± 0.06	0.0303 ± 0.0028	0.0117 ± 0.0014	8.36 ± 0.54	9.67 ± 0.35
Note: Chinese fir plant: leaf, branch, stem, bark, fine root and coarse root of Chinese fir; Litter: litter leaf and branch in L layer and F layer; Understorey layer: small tree, liana, shrub, and herb; Soil: total N storage of three soil layers in 0–20 cm, 20–40 cm, and 40–60 cm; Total in ecosystem: the sum N storage of Chinese fir plant, litter, understorey layer, and soil. N0, control; N60, low-N; N120, medium-N; N240, high-N. The same as below.					

Table 5
Recovery rate of N (%) by vegetation from N fertilizer in the rate of 60,120 and 240 kg hm⁻²a⁻¹

N fertilizer rate (kg N hm ⁻² a ⁻¹)	Chinese fir plant	Litter	Understorey vegetation	Vegetation
N60	9.31	1.98	-8.88	2.41
N120	20.98	3.00	-4.88	19.11
N240	14.88	0.43	-2.65	12.67
Note: Vegetation: the sum of Chinese fir plant + litter and + understorey layer. The same as below.				

Table 6
Loss of N by vegetation after 14 years N fertilizer at the rate of 60,120 and 240 kg N hm⁻²a⁻¹ relative to the control

N fertilizer rate (kg N hm ⁻² a ⁻¹)	N storage in vegetation (kg N hm ⁻²)	Total amount of N fertilizer in 14 years (kg N hm ⁻²)	Loss of N (kg N hm ⁻²)
N60	14.44	840	825.56
N120	229.27	1680	1450.73
N240	304.07	3360	3055.93

Discussion

Tree growth and mortality

- Although the tree mortality rate in the studied forests (2004–2017) was generally low (average 6.21%), it is noteworthy that tree mortality was higher in the treatments with N fertilization, i.e., 8.76% in N120 and 7.32% in N240 (Fig. 2), which indicates that N fertilization may have influenced tree mortality. This is similar to the findings of a study that reported significantly increased mortality after 18 years of N deposition (30 kg N hm⁻²a⁻¹) and a reduction in the overall survival of groundcover and understorey plants by almost 90% after a 5-year seedling experiment (Talhelm et al. 2013). Some studies found that tree mortality was sensitive to N deposition, especially at high levels of nitrate deposition, which led to increased tree mortality rate due to N saturation and soil acidification (Dietze et al. 2011; Lovett and Goodale 2011). Some researches have attributed the increase in tree mortality in N-saturated forests to a decreases in the Ca:Al ratio and Mg:N ratios in the foliage and organic soil layer (Aber et al. 1998; Wallace et al. 2007; Lovett and Goodale 2011). In addition, Fenn et al. (2020) identified a small but significant effect whereby N deposition increased the mortality due to crown damage, and Tian et al. (2018) proposed that this increase in tree mortality may indirectly related to increases in plant disease, changes in soil microbial communities and greater susceptibility to insect and fungal pathogens. The increase in tree mortality observed in this study suggests that N fertilization negatively affected the trees.
- The increase in BAI and annual BAI only occurred after 10–11 years N treatment in this study confirmed that N fertilization led to an increase in relative tree growth (Wallace et al. 2007). The concurrence of mortality and increased growth in surviving trees is considered as a “threshold” phenomenon in N-saturation. The inherent susceptibility of the study site to nitrate leaching and soil acidification in response to N addition, which is mainly regulated by soil and vegetation N uptake capacity, may be the main factor in determining tree growth and mortality (Wallace et al. 2007). These results are in accordance with several N fertilization experiments showing a potential negative response of forest growth at high levels of N fertilization due to soil acidification and nutrient imbalances (Tian et al. 2018; Midolo et al. 2019).

N storage in the ecosystem

Among the N treatments, the N contents differed significantly in the soil and litter but not in the plant organs and understorey layer. The reason for this finding may be that *C. lanceolata* is not N-fixing plant and the N content of this species shows a neutral response to N fertilization (Tian et al. 2018). Experimental results have shown a significant increase in the soil N content with N fertilization up to a threshold (N120, i.e., a rate of 120 kg N hm⁻²a⁻¹), above which no further increase was observed. Tian et al. (2018) reported that the soil N content increased by 19.24–51.49%, which was higher than the average value (5.4%) in subtropical soil based on meta-analysis, while this value can be increased to 26.9% in tropical forests.

- The consistently negative understorey layer N recovery rate could be attributed to decreased species dominance in plant communities in response to higher N fertilizer rates, which is similar to the conclusion that plant total N uptake controls plant dominance (Jiang et al. 2018; Tian et al. 2018). Understorey layer biomass and N uptake significantly decreased with increasing N fertilizer rate in this study were associated with high levels of N fertilization decrease understorey layer diversity. Some similar studies demonstrated that N addition reduced understorey layer biomass and resulted in the replacement of dominant understorey species due to different sensitivities and tolerances with N (Stevens et al. 2010; Talhelm et al. 2013; Zhang et al. 2014; Midolo et al. 2019; Wu et al. 2021).
- Our results show that N fertilization induced a 31.67% increase in the total ecosystem N storage compared to the control. The findings that are most strongly emphasized in the current paper are that the total N storage was up to 1.4 times higher in N-fertilized treatments than in non-fertilized treatments after 14 years and that the majority (approximately 87.64%) of the N in the ecosystem was stored in the soil. Even though the growth of *C. lanceolata* themselves for 14 years has brought about the accumulation of a large amount of N storage, N fertilization still greatly promotes plants N storage. This effect is most obvious in N120 and N240 treatments (Fig. 3). In addition, the total ecosystem N storage in the medium-N treatment appeared to be promoted to a greater extent than that in the other treatments (Table 3). The highest N application was not associated with the highest ecosystem N storage values which only partial support our first hypothesis. The

positive effects of N fertilization on the forest ecosystem could be largely driven by the comprehensive effects of N cycles. However, the negative effects on N storage that can occur as a result of extremely high N fertilization rates. High soil N storage in forest ecosystems and changes in labile soil N factors in response to extremely high N application rates are undeniable, especially when comparisons are made before and after long-term N fertilization (Tian et al. 2010; 2018).

Low N use efficiency after 14 years of N fertilization

The whereabouts of N fertilizer after being applied to ecosystem can be divided into three parts: absorbed by plants, remaining in soil and loss through different mechanisms and several pathways (Ju et al. 2003; Xu and He 2020). In the current research, *C. lanceolata* in this study accounted over 90.55% of the total vegetation N storage; the average percentage of N stored in the soil (0–60 cm) relative to the total ecosystem N storage was 87.64% on average; when compared with the N storage in vegetation and the total amount of N fertilizer for 14 years, we found N loss was estimated to be over 86%. Medium-N showed the highest N recovery rate of N fertilization which do not support our second hypothesis. While our findings did suggest that N recovery rate at extremely high dose of N fertilizer ($240 \text{ kg N hm}^{-2} \text{ a}^{-1}$) did not correspond with the patterns of high N uptake. It is also important to note the low N recovery rate (average 11.39%) and high loss of N (91.86% on average) after N fertilization in this study, which indicate low plant N use efficiency.

Although the current research resulted in novel findings, there were potential limitations in the study design that could be addressed in future research. For example, calculate the N use efficiency without considering an increase or decrease in the soil N pool may lead to biased results (Tian et al. 2010). Vegetation as an important source of active N, the dynamic N storage changes in different vegetation types still have a high uncertainty (Xu et al. 2020). The influence of long-term N fertilization on ecosystem N storage was examined for only one species (*C. lanceolata*) in this study, and it may be necessary to conduct similar studies on more tree species. In addition, more vegetation samples should be collected during different growth seasons and parts (current-year, previous-year, or two-year-old growth) to increase representation and allow generalization of the results. Detrital biomass, mainly from coarse woody debris, is a large N sink in forests (Fisk et al. 2002; Wu et al. 2020), and it is necessary to determine how the mass loss from detritus biomass influences ecosystem N pools. Furthermore, the balance of soil N nutrients is an important factor that affects the utilization of N (Aber et al. 1998; Li et al. 2019a). We could not explore how soil nutrients regulate plant and ecosystem N storage due to the lack of measured soil N resources (i.e., soil available N). We have planned an ongoing study to determine how changes in nutrient availability over time affect ecosystem N storage after long-term N fertilization. Therefore, to determine whether our results are generally applicable and to coniferous plantation management in subtropical regions, future studies should test more tree species and their N storage at different rates and duration of N fertilization.

Conclusion

Our research highlights the importance of considering various plant organs and ecosystem components in accurately estimating ecosystem N storage. The results presented here only partial support our first hypothesis while do not support our second hypothesis. With the increasing application rate, *C. lanceolata* N storage increased while ecosystem N storage did not increase (up to a threshold in medium-N) after 14 years N fertilization. The total ecosystem N storage was 1.1–1.4 times higher than that of the unfertilized ecosystem. The low N recovery rate (11.39%) and high loss of N (91.86%) suggest that long-term N fertilization might induce low ecosystem N use efficiency. Taken together, results presented here suggest that long-term N fertilization may fundamentally alter the N content, N storage and N recovery rate to cycle N in forest ecosystems, which is a plausible phenomenon reflecting low ecosystem N use efficiency in our long-term N field experiment.

Declarations

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Credit Authorship Contribution Statement

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Data curation, Formal analysis, Writing - original draft, Visualization, Conceptualization, Investigation.

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Supervision, Conceptualization, Methodology, Resources.

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Conceptualization, Methodology, Writing-review & editing.

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Writing - review & editing, Investigation.

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Figures

Figure 1

Soil N content (a) and N storage in total (b) and their three soil layers (0-20 cm, 20-40 cm, and 40-60 cm) under different N treatments

Figure 2

Diameter at breast height (DBH) (a), the number of trees at different DBH classes (4 cm interval) (b) after 14-years N treatments. DBH classes can be defined as DBH ≤ 10 cm, 10 < DBH ≤ 14 cm, 14 < DBH ≤ 18 cm, 18 < DBH ≤ 22 cm, 22 < DBH ≤ 26 cm, 26 < DBH ≤ 30 cm, 30 < DBH ≤ 34 cm and 34 < DBH ≤ 38 cm, represent ≤ 10 cm, 10-14 cm, 14-18 cm, 18-22 cm, 22-26 cm, 26-30 cm, 30-34 cm and 34-38 cm. Withered: the numbers of withered trees in different N treatments. Values are the means ± SEs of the three plots. Within each panel, the F-value and P-value are shown based on one-way ANOVA. Lowercase letters indicate significant differences at P ≤ 0.05. N0, control; N60, low-N; N120, medium-N; N240, high-N. The same as below.

Figure 3

Plant N uptake under different N fertilizer rates in 2003 (blue bar), 2017 (red bar) and their increments from 2003 to 2017 (gray bar)

Figure 4

Changes of understorey layer species (a), biomass (b), and N content (c) and N uptake (d) under different N fertilization

Figure 5

Total litter input biomass (a), leaf and branch biomass in L and F layer (b), litter N content (c), and N uptake in total, leaf, and branch (d) under different N fertilization treatments. L-leaf: litter leaf of undecomposed layer; L-branch: litter branch of undecomposed layer; F-leaf: litter leaf of semi-decomposed layer; F-branch: branch of semi-decomposed layer.

Figure 6

N content of major components of Chinese fir plantation ecosystem under different N fertilizer application. Plant: the conclusion of leaf, branch, stem, bark, fine root, and coarse root of Chinese fir.

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