Inorganic and Organic Nitrogen Acquisition by a Fern 
Dicranopteris dichotoma in a Subtropical Forest in South China

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Abstract

The fern Dicranopteris dichotoma is an important pioneer species of the understory in Masson pine (Pinus massoniana) forests growing on acidic soils in the subtropical and tropical China. To improve our understanding of the role of D. dichotoma in nitrogen (N) uptake of these forests, a short-term 15N experiment was conducted at mountain ridge (MR, with low N level) and mountain foot (MF, with high N level). We injected 15N tracers as 15NH4+, 15NO3 or 15N-glycine into the soil surrounding each plant at both MR and MF sites. Three hours after tracer injection, the fern D. dichotoma took up 15NH4+ significantly faster at MF than at MR, but it showed significantly slower uptake of 15NO3 at MF than at MR. Consequently, 15NO3 made greater contribution to the total N uptake (50% to the total N uptake) at MR than at MF, but 15N-glycine only contributed around 11% at both sites. Twenty-four hours after tracer injection, D. dichotoma preferred 15NH4+ (63%) at MR, whereas it preferred 15NO3 (47%) at MF. We concluded that the D. dichotoma responds distinctly in its uptake pattern for three available N species over temporal and spatial scales, but mainly relies on inorganic N species in the subtropical forest. This suggests that the fern employs different strategies to acquire available N which depends on N levels and time.

Introduction

Nitrogen (N) is a major limiting element in many terrestrial ecosystems [15,32]. In the past three decades a large number of plants have been identified to have the capacity to directly take up organic N, mainly in the form of amino acids from soil solution [3,11,21,22]. Therefore, uptake pattern of different N species may be an important mechanism responsible for species coexistence in plant communities[19]. Numerous studies on plant N acquisition of organic and inorganic N species have been conducted in subtropical and tropical forests, most focusing on tree species [1,27,28,29,36,37,38], bryophytes and lichens [6,34] as well as some epiphytes [8,35]. While there was one study investigating N acquisition by a tropical fern [39], little is done to study organic and inorganic N uptake by tropical ferns.

Dicranopteris dichotoma is an important terrestrial fern and is widely distributed in southern China as an early-stage colonizer of acidic and oligotrophic soils [9,40]. D. dichotoma is characterized by rapid clonal growth and often forms a dense understory layer in humid subtropical and tropical forests (Figure 1). Numerous studies have suggested that Dicranopteris species can influence many ecological processes in these forests, such as soil erosion, nutrient cycling, tree regeneration, and plant community succession [5,25,26,41]. Recently, understory removal experiments showed that the Dicranopteris-dominated understory can form favorable soil microclimates and acts as a major driver of soil biota and ecological processes in forest ecosystems [18,40]. However, N acquisition mechanisms of D. dichotoma remain unknown in these tropical forests, and clarification of D. dichotoma N uptake patterns could improve the mechanistic understanding of its role in these subtropical and tropical forests.

To investigate organic and inorganic N acquisition by D. dichotoma, we selected two different habitats in a subtropical forest (Figure 1): one located at the mountain ridge (MR) and the other located at the mountain foot (MF). Compared to the MF site, the MR site is characterized by heavy soil erosion and relatively lower N availability. Numerous studies have demonstrated that plants under high available N levels show higher N uptake rates [7,30].

Therefore, we hypothesized that D. dichotoma would have greater N uptake capacity at high N level than at low N level. Additionally, we hypothesized that D. dichotoma could acquire more nitrate than ammonium and organic N because it is more mobile in soil solution [31]. To test these hypotheses, we conducted a short-term 15N labeling experiment in both D. dichotoma communities with different available N levels.

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Materials and Methods

Study Site

This study was carried out at Xingguo Soil Erosion Observation Station (which belongs to the Institute of Geographic Sciences and Natural Resources, Chinese Academy of Sciences) in Xingguo County (26°30′N, 115°28′E, 80 m above sea level) of Jiangxi Province, southern China, where no additional special permission for the research site was needed given that the site is owned by the institute and a long-term research permission from the local government exists since 1993. Moreover, our study did not involve any endangered or protected species. Two sites were selected along a mountain slope (Figure 1): one was located at the mountain ridge (MR) and the other was located at the foot of the mountain (MF). The understory was dominated by D. dichotoma at both sites. The Pinus massoniana trees at both sites had been planted more than 20 years before the study. In the MR sites, the trees are very small because of heavily eroded soil. In contrast, the trees grow better in the MF site. Soil was classified as loamy Lixisol and very small because of heavily eroded soil. In the MR sites, the trees are more than 20 years before the study.

Inorganic and Organic N Acquisition by Fern

Thirty-six similar sized clusters of D. dichotoma were randomly selected at both sites. They were divided into three groups with each group including 12 individual plants. Each of the groups was labeled either with 15NH4+ (15NH4NO3, 94.4 atom% 15N), 15NO3 (NH415NO3, 98.2 atom% 15N), and 15N-glycine (C2H515NO2, 95.0 atom% 15N), by injecting 10 μg N g−1 dw soil. The tracers were injected into the soil at 3 cm depth in a pattern representing the three points of a triangle, with 5 cm length between points and the plant at the centre of the triangle. Six clusters were harvested 3 h after the tracer was injected. The remaining 6 clusters for each treatment were harvested 24 h after the tracer was injected. These harvested plants were classified into roots and shoots. An additional 6 clusters were injected with the same amount of water following the same pattern and harvested as the control after 24 h. The roots were put into 0.5 mM CaCl2 solution for 30 min. Then, they were rinsed with purified H2O and dried at 75°C. These plant materials were weighed for biomass. Dried roots and shoots were ground to a fine powder using a ball mill (MM200, Retsch, Germany) for the measurements of N content and 15N/14N ratios. Fresh soil (upper 10 cm) was collected for nutrient analysis.

Soil NO3-N and NH4+-N were determined in 0.5 M K2SO4 extracts on an auto-analyzer (AA3, Bran-Luebbe, Germany). Soil glycine concentrations were measured by high performance liquid chromatography (Waters 515, Waters Inc., USA) in the same extracts [23]. Aliquots of ground plant material (about 2 mg) and soil (about 40 mg) were weighed into tin capsules for analysing organic C, total N and 15N/14N ratios using isotope ratio mass spectrometry (IRMS, MAT 253, Finnigan MAT, Germany), with a Flash EA1112 interfaced by ConFlo III to the IRMS. Soil pH was measured using a glass electrode on a 1:2 soil-to-water ratio by weight.

N Uptake Calculation

Atom% excess 15N (APE) was calculated as the atom% 15N difference between plants from 15N treated and from control plants. 15N uptake by plants was estimated by calculating the 15N excess of each plant part (biomass×%N/100×APE/100 for shoot and root individually) and then summing them up and dividing this number by root biomass and expressed as μg 15N g−1 dw root h−1.

The standard errors of means are presented in figures and tables as a variability parameter. T-test was used to compare the

Table 1. Characteristics of topsoil (0–10 cm) of the mountain ridge (MR) and the mountain foot (MF) sites where Dicranopteris dichotoma grows.

|                      | Mountain ridge | Mountain foot |
|----------------------|----------------|---------------|
| Total N (%)          | 0.037±0.003    | 0.075±0.019   |
| Organic carbon (%)   | 0.65±0.10      | 1.54±0.45     |
| C/N                  | 16.85±1.57     | 19.47±1.33    |
| pH (H2O)             | 4.54±0.04      | 4.45±0.04     |
| Soil moisture (%)    | 8.93±0.50*     | 18.30±1.00*   |
| Ammonium (µg N g−1 dw soil) | 5.07±0.61*   | 14.52±0.79*   |
| Nitrate (µg N g−1 dw soil) | 0.09±0.03     | 0.05±0.01     |
| Glycine (µg N g−1 dw soil) | 0.05±0.01*    | 0.12±0.03*    |

Means (±1SE) of six replicates are presented (n = 6). Asterisks indicate significant differences between two sites at P<0.05.

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difference in N uptake rates and soil characteristics between sites. Tukey HSD test was used to compare the contribution of three N species to total N uptake between MR and MF. Three-way ANOVA was performed to test the effects of site, N species and time and their interactions on N uptake rates. Data transformation of Ln (data) was applied to meet preconditions of variance homogeneity and normal distribution before ANOVA analysis. All differences were tested for significance at $P < 0.05$ and all statistical analysis were performed on SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA).

## Results

Concentrations of soil organic C and total N were consistently but not significantly higher at MF than MR (Table 1). Compared to MR, concentrations of $\text{NH}_4^+$ and glycine were significantly higher at MF (Table 1). $\text{NH}_4^+$ was the dominant N species among the three N species, while nitrate and glycine-N concentrations were comparably low at both sites. Total biomass of *D. dichotoma*, including shoots and roots was significantly lower at MR than at MF ($P < 0.05$) (Fig. 2). *D. dichotoma* at MR also had significantly lower ratios of root to shoot than at MF (MR vs MF: 1.15 ± 0.11 vs 1.76 ± 0.17) (Fig. 2).

Results of three-way ANOVA showed significant effects of site, N species and their interactions on N uptake rates but no significant effect of time (Table 2). Three hours after tracer injection, the fern *D. dichotoma* took up $^{15}\text{NH}_4^+$ significantly faster (MF vs MR: 0.65 ± 0.13 vs 0.36 ± 0.04 µg $^{15}\text{N}$ g$^{-1}$ dw root h$^{-1}$), while it took up less $^{15}\text{NO}_3^-$ (MF vs MR: 0.17 ± 0.03 vs 0.44 ± 0.13 µg $^{15}\text{N}$ g$^{-1}$ dw root h$^{-1}$) at MF than at MR. Compared to $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$, *D. dichotoma* showed considerably lower uptake rates for $^{15}$N-glycine (MF vs MR: 0.10 ± 0.03 vs 0.09 ± 0.02 µg $^{15}\text{N}$ g$^{-1}$ dw root h$^{-1}$) without significant differences between sites (Fig. 3).

Twenty-four hours after tracer injection, uptake rates of $^{15}$N-glycine by *D. dichotoma* did not change at both sites (Fig. 3). Surprisingly, uptake rates of $^{15}\text{NH}_4^+$ significantly increased at MR (0.98 ± 0.15 µg $^{15}\text{N}$ g$^{-1}$ dw root h$^{-1}$) while they decreased at MF.

### Table 2. Results of multifactorial ANOVA for the effects of site, N species, time, and their interactions on N uptake rates of *D. dichotoma* in a subtropical forest.

| Source of variation | $F$  | $P$  |
|---------------------|------|------|
| Site                | 5.10 | 0.03 |
| N species           | 69.54| 0.00 |
| Time                | 2.16 | 0.15 |
| Site * N species    | 4.71 | 0.01 |
| Site * Time         | 5.16 | 0.03 |
| N species * Time    | 1.12 | 0.33 |
| Site * N species * Time | 7.72 | 0.00 |

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(0.27±0.04 μg 15N g⁻¹ dw root h⁻¹). By comparison, uptake rates of 15NO₃⁻ at MF remained unchanged at MR, but they significantly increased at MF (Fig. 5, T-test, P = 0.034).

Three hours after tracer injection, the fern *D. dichotoma* preferentially took up 15NO₃⁻ at MR (50% of total N uptake) while it preferentially took up 15NH₄⁺ at MF (69% of total N uptake). By comparison, 15NH₄⁺ contributed 40% and 69% to the total N uptake at MR and at MF, respectively. The contribution of 15NO₃⁻ was significantly higher at MR than at MF. The contribution of 15N-glycine was around 11% to the total N uptake at either of the sites (Fig. 4).

Distinct N uptake pattern of *D. dichotoma* was observed at 24 h after tracer injection. *D. dichotoma* preferred 15NH₄⁺ at MR, which contributed 63% to the total N uptake, while the fern preferred 15NO₃⁻ at MF, which contributed about 47% (Fig. 4). The contribution of 15N-glycine uptake was significantly higher at MF than at MR (14.8% vs 6.6%) (Fig. 4).

**Discussion**

We investigated inorganic and organic N acquisition patterns by the terrestrial fern *D. dichotoma* at two habitats in a subtropical forest using a short-term 15N labeling experiment. A previous study in a tropical forest showed that amino acid uptake of a low-light understory terrestrial fern (*Danaea wendlandii*) reached up to about 330 μg N g⁻¹ dw root h⁻¹ [39]. In this study, the terrestrial fern *D. dichotoma* showed a much lower uptake rate for glycine-N, only around 0.1 μg 15N  g⁻¹ dw root h⁻¹. One possible explanation is that Watkins [39] tested uptake of amino acids using excised roots in solution while this study was performed in the field. Roots likely had more opportunity to take up glycine-N in solution than in soil. Available soil N concentrations will be changed through microbial competition and due to microbial mineralization of this organic tracer. Low glycine-N concentrations in the soil (Table 1) also reflect this situation. Besides, the injected soil volume was certainly smaller as the volume of soil extracted to collect the roots afterwards, i.e. many of the collected roots never came into contact with 15N. Additionally, excised roots may have been more efficient compared to the roots used in this study, which included more inefficient roots not responsible for nutrient uptake. Nonetheless, it has to be noted that excised roots will loose lose uptake potential over time due to C starvation, because the phloem sugar import has been cut off after root excision.

Our hypothesis that *D. dichotoma* would have greater N uptake capacity at high N level than at low N level was partly supported by our data. Three hours after tracer injection, the fern *D. dichotoma* demonstrated faster uptake rates of 15NH₄⁺ at MF than at MR. One possible explanation could be ascribed to good water status at MF (Table 1), which may enhance the diffusion rate of NH₄⁺ from soil solution to root surface [2, 10]. However, 24 h after tracer injection, the fern *D. dichotoma* growing at MR showed higher uptake rates for the dominant N form NH₄⁺. On the basis of the difference in root biomass between MR and MF (Fig. 2), the efficiency of 15NH₄⁺ acquisition by roots at MR is higher than at MF. This indicates that the optimal performance of NH₄⁺ transporters with high affinity on the root surface [12,24,33] could be more important than soil moisture in these soils where NH₄⁺ is the dominant N species. Besides, strong microbial competition for 15NH₄⁺ could be responsible for low uptake rates by roots at MF [13].

Although soil NO₃⁻ concentrations were considerably lower compared to NH₄⁺ (Table 1), the fern *D. dichotoma* showed higher uptake rates for 15NO₃⁻ and its uptake was similar to 15NH₄⁺ uptake rates in some cases (e.g., 3 h after tracer injection at MR and 24 h after tracer injection at MF). One possible explanation is that NO₃⁻ is more mobile in soil solution [31]. These results indicate that our second hypothesis was partly supported by our observations. This could be explained by the fact that NH₄⁺ is the dominant N species in both habitats (Table 1), and therefore the fern *D. dichotoma* prefers NH₄⁺. The preference for NH₄⁺ was also observed in the tropical terrestrial fern (*D. wendlandii*) despite high NO₃⁻ concentration [39].

The contribution of the three N species to the total N uptake strongly relies on time and site (Table 2, Fig. 4). Under low N levels (MR), *D. dichotoma* preferentially take up NO₃⁻ 3 h after tracer injection, but shifted to the dominant N species (NH₄⁺) 24 h after tracer injection. Under high N levels (MF), *D. dichotoma* preferred the dominant N species (NH₄⁺) at 3 h after tracer injection and shifted to NO₃⁻ at 24 h after tracer injection. The distinct uptake pattern for these available N species observed over temporal and spatial scales suggests that the *D. dichotoma* employs different strategies to acquire available N, depending on N levels and time. This could be ascribed to rapid regulation of N uptake.
modulated by a variety of biotic and abiotic factors in addition to tracer \(^{15}\)N dilution of the available soil N pool, such as the carriers of nitrate, ammonium and amino acids located at the surface of the roots [16,17,20]; soil supply rates of available N [4,14]; delivery of N to the rhizosphere through mass flow and diffusion [10]; as well as competition with soil microorganisms [13]. Further investigations should be focused on how interactions between these biotic and abiotic factors affect N uptake by *D. dichotoma* for a better understanding of the underlying mechanisms.

**Supporting Information**

**Table S1** T-test for individual biomass allocation of *Dicranopteris dichotoma* growing at Mountain Ridge (MR) and Mountain Foot (MF) sites. Supplementary data, including the characteristics of top soil (0–10 cm), individual biomass allocation of *Dicranopteris dichotoma*, uptake rate of different N species and their contribution to total N uptake rate. And statistical analysis results are also included inside. (XLS)

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**Author Contributions**

Conceived and designed the experiments: XX Qingkang Li. Performed the experiments: XX Qingkang Li JW ST L. Zhi Qianru Li YS. Contributed reagents/materials/analysis tools: ST L. Zhang Qingkang Li YS. Wrote the paper: XX Qingkang Li.

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