Effects of Different Soils on the Biomass and Photosynthesis of *Rumex nepalensis* in Subalpine Region of Southwestern China

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Abstract: The performance of *Rumex nepalensis*, an important medicinal herb, varies significantly among subalpine grasslands, shrublands and forest ecosystems in southwestern China. Plant-soil feedback is receiving increasing interest as an important driver influencing plant growth and population dynamics. However, the feedback effects of soils from different ecosystems on *R. nepalensis* remain poorly understood. A greenhouse experiment was carried out to identify the effects of different soil sources on the photosynthesis and biomass of *R. nepalensis*. *R. nepalensis* was grown in soils collected from the rooting zones of *R. nepalensis* (a grassland soil, RS treatment), *Hippophae rhamnoides* (a shrub soil, HS treatment), and *Picea asperata* (a forest soil, PS treatment). The chlorophyll contents, net photosynthetic rates, and biomasses of *R. nepalensis* differed significantly among the three soils and followed the order of RS > HS > PS. After soil sterilization, these plant parameters followed the order of RS > PS > HS. The total biomass was 16.5 times higher in sterilized PS than in unsterilized PS, indicating that the existence of soil microbes in *P. asperata* forest ecosystems could strongly inhibit *R. nepalensis* growth. The root to shoot biomass ratio of *R. nepalensis* was the highest in the sterilized PS but the lowest in the unsterilized PS, which showed that soil microbes in PS could change the biomass allocation. Constrained redundancy analysis and path analysis suggested that soil microbes could impact the growth of *R. nepalensis* via the activities of soil extracellular enzymes (e.g., β-1,4-N-acetylglucosaminidase (NAG)) in live soils. The soil total soluble nitrogen concentration might be the main soil factor regulating *R. nepalensis* performance in sterilized soils. Our findings underline the importance of the soil microbes and nitrogen to *R. nepalensis* performance in natural ecosystems and will help to better predict plant population dynamics.

Keywords: soil microbes; extracellular enzymes; biomass allocation; net photosynthetic rate; plant growth; soil nitrogen concentration

1. Introduction

In terrestrial ecosystems, plants can modify soil properties that consequently influence the performance of other conspecific or heterospecific plants; this mechanism is known as “plant–soil feedback” (PSF) [1,2]. PSF can affect plant population dynamics and species coexistence [3–7]. In previous studies, many ecological theories and empirical studies have indicated that plant interspecific competition for resources (e.g., moisture, light and nutrients) [8,9] and polluted environments (e.g., heavy metal contamination) [10–12] could influence plant biochemical characteristics, individual plant fitness and community assembly. However, the feedback effects of different soils on plant fitness have received relatively little attention [7]. Different plant species can coexist in a local area, and their
presence may be determined by different soil microbes and nutrients [13–15]. Therefore, determining the feedback effects of different soil microbes and nutrient properties on plant performance is necessary to understand the interactions between individual plants and to predict plant coexistence, population establishment and persistence [4,16].

Soil nutrients and soil microbes are two main factors that affect plant production, diversity and population development [17,18]. The microbes are ubiquitous in soils of all plant communities and exert profound effects on plant performance directly through mutualistic or pathogenic interactions [19,20] and indirectly by participating in nutrient cycling [21]. Soil nitrogen and phosphorus cycles are catalyzed mostly by the activities of soil extracellular enzymes, which are synthesized mainly by soil microbes [22]. Soil nutrient conditions are governed primarily by soil microbes that may decompose complex organic matter and release mineral elements by secreting extracellular enzymes [23,24]. Consequently, soil microbes and nutrients interact to influence plant performance and population dynamics [25].

Plant physiological and biochemical characteristics, especially their photosynthetic capacities and pigment concentrations, can represent plant fitness and survival and reflect plant responses to different soil conditions [26–29]. For example, a high photosynthetic capacity is conducive to high plant growth rates and biomass accumulation in suitable natural environments [26]. Photosynthetic pigments, including chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids, are essential factors in plant photosynthesis and can be used to monitor photosynthetic events and evaluate the physiological status of plants [30]. Chl a and Chl b are the major light-harvesting photosynthetic pigments in the chloroplast and are of great significance to the photosynthetic physiology and stress physiology of plants [31]. High leaf chlorophyll contents and photosynthetic rates represent plant adaptability in the highly competitive production environment because these characteristics promote plant rapid growth and enable plants to quickly occupy the limited space and resources [32]. Soil microbes and nutrients can influence plant photosynthetic performance, growth rates and yield [33–35]. At present, studies on PSF focus mainly on the accumulation of plant biomass [2,36,37]. However, little is known about how PSF affects plant performance from the perspective of plant physiological characteristics.

The subalpine region of southwestern China is considered to be a hot spot of biodiversity, where natural ecosystems were damaged by deforestation due to the increased demand of the timber market for materials during the last century [38]. In an effort to revive and restore the forest landscapes in this zone, China conducted reforestation projects in the 1950s and subsequently launched the Natural Forest Protection Program (NFPP) in 2000. Therefore, many different grass, shrub and tree patches were formed within relatively short distances (scales of hundreds to thousands of meters) in this area. *Rumex nepalensis* is a perennial herb with large roots, erect stems and a height of 50–100 cm. It is widely distributed throughout the subalpine region of southwestern China and is well known to be an important medicinal herb [39]. *R. nepalensis* is dominant in some grasslands and coexists with *Hippophae rhamnoides* in shrublands, whereas it cannot grow in the understory of mature *Picea asperata* forests. This phenomenon is generally believed to result from plant interspecific competition for resources (e.g., moisture, light and nutrients) [8,9], but the feedback effects of the different soil parameters on *R. nepalensis* growth in these ecosystems remain unclear.

To enhance our understanding of the distinct performances of *R. nepalensis* in subalpine ecosystems from the perspective of PSF, we conducted a pot culture experiment with rooting zone soils collected from three dominant plant species located in different ecosystems and corresponding sterilized soils. The effects of different soils on the photosynthesis, biomass, and biomass allocation of *R. nepalensis* were investigated. We tested the hypotheses that (1) *R. nepalensis* photosynthesis, biomass and biomass allocation would differ significantly among soils characterized by different PSF effects and (2) the soil microbes of the *P. asperata*
rooting zone would inhibit the growth of *R. nepalensis*, as indicated by the absence of *R. nepalensis* in the understory of mature *P. asperata* forests.

2. Materials and Methods

2.1. Soil and Seed Collection

In October 2018, based on a previous field investigation, we selected three different life-form plant species (*R. nepalensis*, a perennial herb; *H. rhamnoides*, a widespread shrub; and *P. asperata*, a dominant tree) from grasslands, shrublands and forest ecosystems, respectively, in the subalpine region of southwestern China (31°37′–47′ N, 102°41′–50′ E, and 2966–3234 m a.s.l.), for soil collection. These species are widely distributed and easily found in the study area. The soil samples (0–20 cm depth) were collected from the rooting zones (<0.2 m from the base of the stem of *R. nepalensis*; <0.5 m from the base of the stem of *H. rhamnoides*; and <1 m from the base of the stem of *P. asperata*) of 20–30 mature individual plants of each species. The samples were transported back to the lab and pooled. Large rocks and roots were removed from the soils, and the soils were sieved using a 5 mm mesh, packaged in separate polypropylene woven bags and stored in a cool (<17°C), dark room. Seeds of *R. nepalensis* were collected from a population near the fields where the soils were collected.

2.2. Greenhouse Experiment

The greenhouse pot experiment was conducted in a laboratory at Chengdu Institute of Biology, Chinese Academy of Sciences. To ensure good soil aeration and drainage, we added 30% quartz sand (w/w), sterilized by autoclaving at 121°C for 30 min) to all soils based on the ratio of stones to soil after sieving. The field-collected soils of each soil origin (i.e., from beneath each plant species) were homogenized separately. The soil samples were divided into two parts: one was used directly in the subsequent pot experiments, and the other was sterilized by triple-steam pasteurization at 80°C for two hours every two days for six days [37]. This sterilization approach can have fewer side effects on soil chemical and physical properties than other sterilization methods [40], and the key chemical properties of our soils were broadly unaffected (Table 1). The *R. nepalensis* seeds were surface-sterilized with a 0.5% (v/v) potassium permanganate solution for 30 min before rinsing three times with sterile deionized water prior to sowing.

Table 1. Soil chemical properties of the three different unsterilized and sterilized soils used in the glasshouse experiment (mean ± 1 SE, n = 5).

| Soil Properties | Unsterilized Soil | Sterilized Soil |
|-----------------|-------------------|-----------------|
|                 | RS                | HS              | PS              | RS                | HS              | PS              |
| SOC (g kg⁻¹)    | 38.30 ± 0.19 a    | 25.67 ± 0.60 c  | 35.23 ± 0.05 b  | 37.94 ± 0.34 a    | 24.38 ± 0.22 c  | 34.95 ± 0.06 b  |
| TN (g kg⁻¹)     | 3.70 ± 0.04 a     | 2.80 ± 0.05 b   | 2.59 ± 0.06 b   | 3.76 ± 0.02 a     | 2.83 ± 0.05 b   | 2.62 ± 0.03 c   |
| TP (g kg⁻¹)     | 1.018 ± 0.006 aA  | 0.660 ± 0.007 b | 0.650±0.005 bA  | 0.988 ± 0.007 aB  | 0.676 ± 0.007 b | 0.668 ± 0.004 bB|
| SAP (mg kg⁻¹)   | 14.35 ± 0.14 aA   | 5.23 ± 0.14 bA  | 3.02 ± 0.05 cA  | 15.34 ± 0.41 aB   | 5.66 ± 0.06 bB  | 3.41 ± 0.09 cB  |

RS, *R. nepalensis* soil; HS, *H. rhamnoides* soil; PS, *P. asperata* soil; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; SAP, soil available phosphorus. Different lowercase letters in the same row indicate statistically significant differences among the three different soils in the same treatment (p < 0.05). Different uppercase letters in the same row indicate statistically significant differences between unsterilized and sterilized soils of the same type (p < 0.05).

The pot culture experiment was established in the following treatment combinations: three soil origins (herb, shrub, tree) × two soil treatments (sterile, nonsterile). Each treatment combination was replicated ten times, resulting in a total of 60 individual pots (11 cm high, 12 cm in diameter). All soil-collection and potting equipment was cleaned and then sterilized with 75% (v/v) ethanol between soil treatment combinations to avoid cross-contamination. To ensure seedling establishment, the soil was well watered before sowing, and seeds of *R. nepalensis* were sown into holes in the soil. Weeds and grasses were manually removed from the pots to eliminate the potential effects of other plants. The pots were watered with deionized water every two days and weighed regularly in order to...
keep soil moisture content at the 40%–50% (w/w) level. The pots were arranged randomly on glasshouse benches and were rotated periodically to reduce the effects of potential variations in the glasshouse microclimate. The glasshouse conditions were maintained such that the air humidity was maintained from 75%–80%. The daytime temperatures ranged from 20 to 24 °C, and the nighttime temperatures ranged from 16 to 20 °C. A constant photoperiod was applied throughout the experiment using supplemental growth lights to ensure 10 h of daylight, in accordance with the light period in the field. After a 4-month growth period, all seedlings of *R. nepalensis* were harvested.

### 2.3. Photosynthesis and Chlorophyll Measurements

Five pots were randomly selected from each soil treatment. Two leaves from the different plants in each pot were chosen and signed for the measurements. The open gas-exchange system (LI-6400XT, Li-Cor Inc., Lincoln, NE, USA) is used to measure the net photosynthetic rate (Pn) of the leaves between 08:30 and 11:30 in the morning. The saturating photon flux density was set at 800 µmol m⁻² s⁻¹. The conditions in the leaf chamber were similar to the growth conditions and maintained at 20–23 °C, about 25% relative humidity and 380–400 µmol mol⁻¹ ambient CO₂ concentration. Before measurement, the leaves were placed in the leaf chamber for 5 minutes to gain photosynthetic stability. Measurements were made in triplicate from the two previously labeled leaves. At the end of the Pn measurements, the marked leaves were excised for determinations of Chl a, Chl b and Chl a + b in fresh leaves using the method described by Inskeep and Bloom [41].

### 2.4. Plant and Soil Sampling

All *R. nepalensis* plants were harvested and separated into their root and shoot parts. All plant samples were washed with tap water and dried at 80 °C to constant weights. The root to shoot biomass ratio (g root/g shoot plant) were calculated on the basis of the values from the measured plants. All rhizosphere soil samples were carefully collected by collecting all the soil adhering to the roots after gentle shaking; the samples were then homogenized and passed through a 1-mm-mesh sieve. Each soil sample was divided into three subsamples: one was stored at −20 °C for microbial biomass analysis and enzyme activity analysis, one was stored at 4 °C for available nutrient analysis, and one was air-dried and sieved using a 0.25 mm mesh for other chemical analysis.

### 2.5. Soil Property Analysis

The soil organic carbon (SOC) concentration was determined using H₂SO₄-K₂Cr₂O₇ oxidation, and the soil total nitrogen (TN) concentration was measured with a C/N elemental analyzer (Multi-N/C 2100, Analytik Jena AG, Jena, Germany). The soil total phosphorus (TP) concentration was measured using an inductively coupled plasma–atomic emission spectrometer (ICP-AES Optima 8300, PerkinElmer, Waltham, American) after digestion with H₂SO₄ and HClO₄ solution [42], and soil available phosphorus (SAP) was extracted with Bray-I solution [43] and then measured using the same ICP-AES method used for TP determination. Soil ammonium nitrogen (AN) and nitrate nitrogen (NN) were extracted by 2 M KCl (soil: solution = 1:5) and then determined on a continuous flow injection analyzer (SEAL Analytical, Norderstedt, Germany). Soil total soluble nitrogen (TSN) was measured using the same apparatus used for AN measurement. The concentrations of soil microbial biomass carbon, nitrogen and phosphorus (SMBC, SMBN and SMBP) were determined by the chloroform fumigation method [44]. The potential activities of the primary soil organic carbon-, nitrogen- and phosphorus-degrading enzymes (β-1,4-glucosidase (BG), β-N-acetylglucosaminidase (NAG), and acid phosphatase (AP)) were determined using a fluorometric microplate assay with a 4-methylumbelliferylsubstrate conjugate [45]. Soil pH was measured with a pH electrode (FE28-Meter, Mettler Toledo, Zurich, Switzerland) in a suspension with a 1:2.5 (w/v) ratio of soil to deionized water.
2.6. Statistical Analysis

One-way analysis of variance (ANOVA) with the least significant difference (LSD) test (significant at the \( p < 0.05 \) level) for post hoc multiple comparisons was applied to examine the effects of three different soils on \( \text{Pn} \), chlorophyll concentrations, biomass and biomass allocation in \( R. \text{nepalensis} \). Independent-samples \( t \)-test was used to compare the mean values of the plant traits in unsterilized soil vs. sterilized soil. If the data were heterogeneous, they were transformed to meet the assumptions necessary for ANOVA prior to the analysis. To test whether the soil microbes and nutrient properties influenced the chlorophyll concentrations, \( \text{Pn} \), biomass or biomass allocation of \( R. \text{nepalensis} \), redundancy analysis (RDA) was carried out with CANOCO 5.0 (Biometris, Wageningen, The Netherlands). We constructed a structural equation model (SEM) using AMOS 22 (SPSS Inc., Chicago, IL, USA) to examine how soil properties affect the performance of \( R. \text{nepalensis} \) in unsterilized soils. The following indices were used to verify whether our data conformed to the model: Chi-square/df (Normed chi-square, NC) \(< 2\), \( p \) values > 0.05; goodness of fit index (GFI) > 0.90 and root mean square error of approximation (RMSEA) < 0.05 [46]. The basic analyses above were performed and the graphics were created mainly in the ‘vegan’ and ‘ggplot2’ packages in R4.0.0 [47].

3. Results

3.1. Soil Properties

Initially, the SOC, TN and TP of the unsterilized soils from the different rooting zones were roughly similar to those of the sterilized soils; only SAP increased slightly after sterilization (Table 1). At the end of the experiment, the SOC, TN, TSN, NN, AN and SAP were significantly lower in sterilized soils than in unsterilized soils (Table 2), especially for the available N levels (i.e., TSN, NN and AN). In unsterilized soils, SMBC and NAG activity were the highest in the PS treatment and the lowest in the RS treatment.

Table 2. Soil properties of the three different unsterilized and sterilized soils after 4 months of \( R. \text{nepalensis} \) growth (mean \( \pm 1 \) SE, \( n = 5 \)).

| Soil Properties | Unsterilized Soil | Sterilized Soil |
|-----------------|------------------|-----------------|
|                 | RS               | HS              | PS              | RS               | HS              | PS              |
| SOC (g kg\(^{-1}\)) | 39.13 \( \pm 0.45 \) aA | 26.8 \( \pm 0.21 \) cA | 35.4 \( \pm 0.02 \) bA | 36.94 \( \pm 0.17 \) aB | 24.09 \( \pm 0.07 \) cB | 31.96 \( \pm 0.39 \) bB |
| TN (g kg\(^{-1}\)) | 3.96 \( \pm 0.04 \) aA | 3.01 \( \pm 0.02 \) bA | 3.11 \( \pm 0.03 \) bA | 3.81 \( \pm 0.04 \) aB | 2.94 \( \pm 0.03 \) bB | 2.72 \( \pm 0.02 \) cB |
| TP (g kg\(^{-1}\)) | 0.840 \( \pm 0.007 \) a | 0.530 \( \pm 0.004 \) cA | 0.556 \( \pm 0.005 \) bA | 0.814 \( \pm 0.013 \) aA | 0.572 \( \pm 0.011 \) bB | 0.518 \( \pm 0.004 \) cB |
| DTN (mg kg\(^{-1}\)) | 17.23 \( \pm 0.36 \) bA | 10.32 \( \pm 0.33 \) cA | 21.14 \( \pm 0.69 \) aA | 10.65 \( \pm 0.44 \) bB | 6.10 \( \pm 0.13 \) bB | 6.44 \( \pm 0.23 \) bB |
| NN (mg kg\(^{-1}\)) | 10.55 \( \pm 0.48 \) bA | 7.74 \( \pm 0.48 \) cA | 15.94 \( \pm 1.13 \) aA | 2.74 \( \pm 0.27 \) aB | 1.50 \( \pm 0.16 \) bB | 2.26 \( \pm 0.20 \) aB |
| AN (mg kg\(^{-1}\)) | 3.32 \( \pm 0.14 \) bA | 2.65 \( \pm 0.06 \) cA | 4.25 \( \pm 0.11 \) aA | 1.82 \( \pm 0.11 \) bB | 1.47 \( \pm 0.02 \) bB | 1.48 \( \pm 0.04 \) bB |
| SAP (mg kg\(^{-1}\)) | 10.03 \( \pm 0.26 \) aA | 5.06 \( \pm 0.07 \) bA | 2.63 \( \pm 0.07 \) cA | 9.06 \( \pm 0.15 \) aB | 3.59 \( \pm 0.04 \) bB | 1.79 \( \pm 0.09 \) cB |
| SMBB (mg kg\(^{-1}\)) | 230.71 \( \pm 10.30 \) c | 303.18 \( \pm 11.05 \) b | 423.16 \( \pm 8.79 \) a | ND | ND | ND |
| SMBN (mg kg\(^{-1}\)) | 53.92 \( \pm 2.95 \) b | 72.31 \( \pm 3.08 \) a | 80.51 \( \pm 3.92 \) a | ND | ND | ND |
| SMBP (mg kg\(^{-1}\)) | 24.68 \( \pm 0.51 \) a | 9.23 \( \pm 0.43 \) c | 13.19 \( \pm 0.86 \) b | ND | ND | ND |
| BG (nmol h\(^{-1}\) g\(^{-1}\)) | 186.15 \( \pm 7.11 \) a | 122.07 \( \pm 6.33 \) b | 199.80 \( \pm 4.95 \) a | ND | ND | ND |
| NAG (nmol h\(^{-1}\) g\(^{-1}\)) | 37.79 \( \pm 2.80 \) c | 66.87 \( \pm 3.87 \) b | 255.20 \( \pm 4.82 \) a | ND | ND | ND |
| AP (nmol h\(^{-1}\) g\(^{-1}\)) | 151.75 \( \pm 6.27 \) a | 97.55 \( \pm 2.95 \) c | 124.96 \( \pm 3.58 \) b | ND | ND | ND |

RS, \( R. \text{nepalensis} \) soil; HS, \( H. \text{rhamnoides} \) soil; PS, \( P. \text{asperata} \) soil; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; SAP, soil available phosphorus; TSN, soil total soluble nitrogen; NN, soil nitrate nitrogen; AN, soil ammonium nitrogen; BG, \( \beta \)-1,4-glucosidase; NAG, \( \beta \)-1,4-N-acetylglucosaminidase; AP, acid phosphatase; SMBC, soil microbial biomass carbon; SMBN, soil microbial biomass nitrogen; SMBP, soil microbial biomass phosphorus; ND, no data/not measured. Different lowercase letters in the same row indicate statistically significant differences among three different soils in the same treatment (\( p < 0.05 \)). Different uppercase letters in the same row indicate statistically significant differences between unsterilized and sterilized soils of the same type (\( p < 0.05 \)).

3.2. Biomass and Biomass Allocation

The root, shoot, and total biomasses of \( R. \text{nepalensis} \) in the different unsterilized soils followed the order of RS > HS > PS, respectively, while in the sterilized soils, they were ranked as RS > PS > HS (Figure 1a–c). The total biomass of \( R. \text{nepalensis} \) in the unsterilized
RS was approximately 19.8 times that in the unsterilized PS and 16.5 times higher in the sterilized PS than in the unsterilized PS ($p < 0.001$) (Figure 1c). The root to shoot biomass ratio were the highest in the sterilized PS and the lowest in the unsterilized PS, and showed a significant response to the soil sterilization treatment in RS and PS ($p < 0.05$, Figure 1d).

![Figure 1](image-url)

**Figure 1.** Effects of different soils on plant biomass and biomass allocation in *R. nepalensis*. (a) root biomass; (b) shoot biomass; (c) total biomass; (d) root to shoot biomass. Bars show the mean values; error bars represent ± SEs (n = 5). Different letters above the error bars indicate significant differences ($p < 0.05$) among the various soil types (RS, *R. nepalensis* soil; HS, *H. rhamnoides* soil; PS, *P. asperata* soil). Asterisks under the bars represent significant differences in plant growth in nonsterile (NS) versus sterile (ST) soil according to a *t*-test (*, 0.01 < $p$ < 0.05; **, $p < 0.01$; ***, $p < 0.001$).

### 3.3. Photosynthesis and Chlorophyll

In the unsterilized soils, the Chl a, Chl a + b and Pn of *R. nepalensis* differed significantly among the three different soils ($p < 0.05$), ranking RS > HS > PS (Figure 2a–c). Chl b was significantly higher in RS and HS than in PS (Figure 2b). In the sterilized soils, the Chl a, Chl b and Chl a + b were the highest in RS ($p < 0.05$) and were significantly higher than those in HS and PS (Figure 2a–c). The Chl a, Chl a + b and Pn of *R. nepalensis* in PS were remarkably enhanced by soil sterilization treatment ($p < 0.001$, Figure 2a–d).
Figure 2. Effects of different soils on plant chlorophylls in fresh leaves and net photosynthetic rate of R. nepalensis. (a) Chlorophyll a; (b) Chlorophyll b; (c) Chlorophyll a + b; (d) Net photosynthetic rate. Bars show the mean values; error bars represent ± SEs (n = 5). Different letters above the error bars indicate significant differences (p < 0.05) among the various soil types. Asterisks under the bars represent significant differences in plant growth in nonsterile (NS) versus sterile (ST) soil according to a t-test (*, 0.01 < p < 0.05; ***, p < 0.001).

3.4. Effects of Soil Factors on Plant Performance

The constrained RDA indicated that the constraining variables accounted for 97.5% and 47.0% of the total variances in the unsterilized and sterilized soils, respectively. In the unsterilized soils, NAG activity and SMBC were two main soil factors affecting plant performance, accounting for 79.9% and 79.0% of the total variance (simple effect, p = 0.002), respectively (Figure 3a). However, the TSN concentration was the most significant variable affecting plant performance, accounting for 31.2% of the total variance in the sterilized soils (Figure 3b).

The model based on all indicators fully fitted the data describing the impacts of soil properties on plant performance ($\chi^2 = 2.610$, $p = 0.625$, NC = 0.652, RMSEA < 0.001, GFI = 0.941; Figure 4) in the unsterilized soils, which accounted for 85% of the variation in shoot biomass and 78% of the variation in root biomass and showed that SMBC could directly influence the root biomass and indirectly impact the Pn and shoot biomass via soil NAG activity.
Figure 3. Bidimensional graph of the redundancy analysis, indicating the relationships between plant growth indexes and soil parameters, in the unsterilized soils (a) and sterilized soils (b). SB, shoot biomass; RB, root biomass; TB, total biomass; R/S, root to shoot biomass; Pn, net photosynthetic rate; Chl a, chlorophyll a; Chl b, chlorophyll b; Chl a + b, chlorophyll a + b; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; SAP, soil available phosphorus; TSN, soil total soluble nitrogen; NN, soil nitrate nitrogen; AN, soil ammonium nitrogen; BG, β-1,4-glucosidase; NAG, β-1,4-N-acetylglucosaminidase; AP, acid phosphatase; SMBC, soil microbial biomass carbon; SMBN, soil microbial biomass nitrogen; SMBP, soil microbial biomass phosphorus.
were the highest in RS and the lowest in PS. These results are consistent with our first hypothesis that the different soils would differentially affect R. nepalensis performance, which may provide insights into the different performance of R. nepalensis across grasslands, shrublands and forests.

4.1. Feedback Effects of Different Soils on Plant Biomass and Physiology

Among the unsterilized soils, the photosynthesis indices, and biomass of R. nepalensis were the highest in RS and the lowest in PS. These results are consistent with our first hypothesis that the different soils would differentially affect R. nepalensis photosynthesis, biomass and biomass allocation. The overall inhibitory effect of various soils on R. nepalensis performance would significantly become enhanced with the increase in soil microbial biomass from grassland, shrubland to forest [25]. The total biomass of R. nepalensis increased to some extent after soil sterilization; most notably, in PS. Therefore, our results could indicate that the inhibitory effect of soil microbes was removed after soil sterilization [48,49]. In the unsterilized PS, SMBC was the highest, but the biomass of R. nepalensis was the lowest. This response was probably due to the existence of large amount of harmful soil organisms (especially for the soil pathogens and root herbivores) in live PS [25,49], which may partially explain the fact that R. nepalensis does not grow in the understory of natural mature P. asperata forests.

Biomass allocation refers to the distribution of dry matter accumulated within a period of time to each plant part and is usually influenced by the growth environment, evolutionary history and interplant competition; these factors may directly affect the ability of plants to adapt to the environment [40–52]. The root: shoot (below: above ground) biomass ratio (R/S) is generally used to characterize changes of photosynthetic product distribution pattern and could most directly reflect biomass allocation by plants [53,54].
The R/S was the lowest in the unsterilized PS and the highest in the sterilized PS, which indicated that soil microbes in PS might have a significant inhibiting effect on root growth of *R. nepalensis*. There are several possible explanations for this. First, large amounts of root herbivores (e.g., root-feeding nematodes) may exist in the unsterilized PS and damage more plant roots, thereby dramatically reducing the R/S of *R. nepalensis* [55–57]. Secondly, *R. nepalensis* plant may tend to form weak associations with soil mutualists and be more susceptible to pathogens in the unsterilized PS [48,58]. Overall, the R/S of *R. nepalensis* showed different responses to the soil treatments, supporting our hypothesis that different soils make plant biomass allocation strategies diverse.

Photosynthesis is a most important metabolic process of plants, which is a sensitive indicator in reflecting changes of external environment [53]. Chlorophylls, including Chl a, Chl b and Chl a + b, are vital indicators of plant photosynthesis and can indicate the ability of plants to convert photosynthetic energy into biomass [59]. Differences in Pn and chlorophyll levels are often used to measure plant responses to different environmental conditions and plant stress resistance mechanisms [60]. In the unsterilized soils, Chl a, Chl b, Chl a + b and Pn in *R. nepalensis* differed markedly among the three different soils and were the lowest in PS. These results suggest that *R. nepalensis* growth would be greatly suppressed in the understory of natural mature *P. asperata* forests in these soil environments, consistent with our second hypothesis. Moreover, these indices related to plant photosynthesis had higher values in the sterilized soils than in the unsterilized soils, suggesting that the soil microbe could affect the photosynthesis capability of *R. nepalensis*, and corresponding plant productivity [61,62].

### 4.2. Important Soil Parameters Affecting Plant Performance

In the sterilized soils, differences were observed in the performance of *R. nepalensis* in various soils with distinct soil nutrient contents. The constrained RDA indicated that TSN concentration was a significant soil nutrient index influencing the biomass of *R. nepalensis* in the sterilized soils. The nitrogen is one of the basic elements of metabolism in nature. Plants allocate more nitrogen to leaves in order to synthesize more chlorophyll to increase plant photosynthesis and achieve faster growth, and even small changes in nitrogen allocations would immensely affect Pn and thereby plant growth [63,64]. In addition, the nitrogen was usually considered a major limiting element for plant growth in subalpine terrestrial ecosystems of southwestern China [65,66]. It is absorbed by plants as dissolved nitrogen in soil, including as dissolved organic nitrogen and dissolved inorganic nitrogen [67–69]. Therefore, soil TSN concentration was an extremely important soil parameter influencing the performance of *R. nepalensis* in the sterilized soils.

Both constrained RDA and path analysis suggested that SMNC and NAG activity were the main soil factors affecting the performance of *R. nepalensis* in the unsterilized soils, and the path analysis indicated that soil microbes could not only directly affect the root and shoot biomass but could also indirectly impact the photosynthesis and shoot biomass via the activities of extracellular enzymes (e.g., NAG). Soil extracellular enzymes are released mainly by soil microbes and participate in soil nutrient cycling [22,70]; for example, NAG degrades chitin and other β-1,4 glucosamine polymers as part of the nitrogen cycle [71]. Soil microbes are superior competitors for organic and inorganic nitrogen in some natural contexts compared with plant roots because of their high surface area:volume ratios and rapid growth rates [72–74]. The SMBC level was the highest in the unsterilized PS, which suggested that competition between plants and soil microbes for nitrogen was more intense in this soil than in the other soils [21] and that the unsterilized PS might contain more microbes that inhibit the growth of *R. nepalensis*. Therefore, soil microbes may directly affect the performance of *R. nepalensis* via some soil pathogens and root herbivores; furthermore, soil microbes could compete for nitrogen via NAG activity and reduced the absorption of nitrogen by plants, thereby affecting the photosynthesis and growth of *R. nepalensis*.
5. Conclusions

In conclusion, our results show that the growth of *R. nepalensis* was inhibited by soil microbes from the rooting zone soils of different plants, especially in the live PS. Soil TSN content was the significant soil nutrient factor influencing the performance of *R. nepalensis* in the sterilized soils, and SMBC and NAG were the most important soil factors influencing *R. nepalensis* growth in the unsterilized soils. These results suggest that appropriate soil sterilization and nitrogen fertilizer application would benefit the establishment of *R. nepalensis* populations. Therefore, the feedback effects of different soil microbes on plant performance should be considered during *R. nepalensis* population development. Further studies exploring the different functional groups of soil microbes and the causal mechanisms regulating plant–soil microbe interactions are needed.

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