Rhizosphere Effects along an Altitudinal Gradient of the Changbai Mountain, China

Changfu Huo 1, Jiayu Lu 1, Liming Yin 1, Peng Wang 1,* and Weixin Cheng 2

1 Key Laboratory of Forest Ecology and Management (CAS), Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China; chuo@iae.ac.cn (C.H.); jiayulu@iae.ac.cn (J.L.); limingyin@iae.ac.cn (L.Y.)
2 Environmental Studies Department, University of California, Santa Cruz, CA 95064, USA; wxcheng@ucsc.edu

* Correspondence: wangpeng@iae.ac.cn; Tel.: +86-24-83970452

Abstract: Rhizosphere effects (REs) play important roles in regulating carbon (C) and nutrient cycling in terrestrial ecosystems. However, little is known about the REs of mature trees in the field, especially at the ecosystem scale. This study aimed to explore the variation and patterns of REs in natural ecosystems. Here, combining soil monoliths with an adhering soil (shaking fine roots) method was adopted to sample paired rhizosphere soil and bulk soil along an altitudinal gradient. Based on the relative REs and the percentage of rhizosphere soil mass, the REs on soil C and net nitrogen mineralization rates (C\text{min} and net N\text{min}) at the ecosystem scale were estimated. Our results showed that the REs on soil processes, soil microbial biomass C and extracellular enzyme activities (\(\beta\)-glucosidase and N-acetyl-glucosaminidase activities), and soil chemical properties (total C, total N, inorganic N, extractable P, K, Ca, Mg, Fe, and Mn) were significantly positive across altitudinal sites, while soil pH was significantly negative. Although the relative REs on investigated variables varied significantly among altitudes, the relative REs did not show a clear trend with the increased altitudes. Across altitudes, the mean magnitude of ecosystem-level REs on C\text{min} and net N\text{min} were 19% (ranging from 4% to 48%) and 16% (ranging from 3% to 34%), respectively. Furthermore, the magnitude of ecosystem-level rhizosphere effects increased linearly with the increased altitudes. The altitudinal patterns of ecosystem-level RE mainly depend on the percentage of rhizosphere soil mass. In conclusion, our results provided a set of new evidence for the REs, and highlighted the need to incorporate REs into land C and N models.

Keywords: bulk soil; elevation; soil carbon mineralization; net nitrogen mineralization; rhizosphere priming; rhizosphere soil mass

1. Introduction

Plant roots not only absorb water and nutrients from soil, but also release a variety of rhizodeposits to the soil [1,2]. Compared with bulk soil, the rhizosphere soil has substantial differences in soil physical and chemical properties, microbial community and activity, and carbon (C) and nutrient cycling [3,4]. This phenomenon is defined as rhizosphere effect (RE) [5]. It is increasingly being recognized that REs govern biogeochemical cycling of many elements in terrestrial ecosystems [6,7]. Recently, meta-analysis studies showed that the mean magnitude of REs on soil C and nitrogen (N) mineralization rates ranged from 22% to 82% [8-10]. However, our understanding of RE is still insufficient, which limits our ability to incorporate the RE into terrestrial C and N models.

Some studies examined the RE of mature trees in the field [10]. Sampling paired rhizosphere soil (adhering soil to the fine roots) and bulk soil is a practical method to explore the in situ REs of forests [5,11]. Based on the adhering soil method, previous studies reported the REs on many soil variables including soil C and net N mineralization rate [12,13], microbial biomass and enzyme activities [12,14,15], and soil nutrient content [16,17]. Most of these studies on the REs of mature trees focused on the differences between tree species [18].
REs are likely to vary with tree species and ecosystem types [19–21]. Notably, few studies evaluated the ecological relevance of REs on C and N mineralization in plantations [5] and a deciduous hardwood forest [22]. Therefore, in order to comprehensively evaluate the ecological relevance of REs, we need to carry out research in more terrestrial ecosystems, especially in natural forests.

Altitudinal gradient is an optimal way to explore the variation and pattern of rhizosphere effects. Such gradients represent powerful “natural experiments” to gain insights into the response of REs to variations in climate and biotic characteristics over short distance [23]. Extensive studies reported that many variables such as soil C and N mineralization rate, soil nutrients, and microbial community structure systematically changed along altitudinal gradients [24–26]. These studies suggested that REs may vary with altitude. Recently, a study reported that the difference in the microbial extracellular enzyme activities between rhizosphere and bulk soil diminished as the altitude increased [27]. Moreover, the responses of REs on soil C and phosphorous (P) availability to high and low altitudes were different [28,29]. Thus far, the effect of altitude on RE is rarely reported, and the pattern of RE along altitude gradients is still unknown.

Changbai Mountain is famous for its greatly vertical distribution of vegetation types, from temperate forests at the bottom to the alpine tundra. The diverse ecosystems provide an ideal place to explore the RE in natural ecosystems. The objectives of this study were: (1) to reveal the patterns of REs on soil assays along an altitudinal gradients and (2) to explore the magnitude of RE on soil C and N mineralization at the ecosystem scale. To our knowledge, this study is the first to elucidate the ecosystem-level REs along an altitudinal gradient, which might provide insights into belowground C and N cycling.

2. Materials and Methods

2.1. Study Area

We performed our sampling in the Changbai Mountains National Natural Reserve, Jilin province, northeast China (41°26′–42°43′ N, 127°17′–128°42′ E). The Changbai Mountain is a dormant volcano, and the last eruption (1702) almost destroyed the vegetation on the mountain. There is little human disturbance in the core zone of the reserve. A diverse natural forest and alpine tundra ecosystems are well-developed along the great span in altitude (530–2749 m). The vegetation is vertically divided into four zones [30]: (1) a mixed coniferous and broad-leaved forest zone (<1100 m) dominated by Pinus koraiensis, Quercus mongolica, Tilia amurensis, Fraxinus mandshurica, and Populus davidiana, where soil is an Albi-Boric Argosol, (2) a dark coniferous forest zone (1100–1800 m) dominated by Picea jezoensis and Abies nephrolepis, where soil is a Bori-Udic Cambosol, (3) an Ermans birch forest zone (1800–2000 m) dominated by Betula ermanii and Larix olgensis, where soil is a Umbri-Gelic Cambosol, and (4) an alpine tundra zone (>2000 m) dominated by Rhododendron chrysanthum, Alnus mandshurica, and Vaccinium uliginosum, where the soil is formed on the rock layer of the permafrost Cambisol. The climate is a typical continental temperate monsoon climate, with a dry and windy spring, a short and rainy summer, a cool and foggy autumn, and long, cold winters. With the increasing altitudes from 700 to 2600 m, mean annual temperature decreases from 2.8 to −6.9 °C, and mean annual precipitation increases from 679 to 1330 mm [31].

2.2. Soil Sampling

In July 2019, we sampled soils along an altitudinal gradient from 800 to 2200 m, with the altitudinal interval approximately 100 m. Since the slight change in vegetation composition and gentle slope below 1300 m, we set only one sampling site in this area. A total of eleven sampling altitudes (800, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, and 2200 m) were set. We randomly selected four plots (20 m × 20 m) in each altitudinal site. In each plot, we randomly select three points for collecting top layer (0–10 cm) mineral soil by soil monolith (30 width × 30 length × 10 depth cm) and pooled to form one composite sample. The soil monoliths were placed into a sorting plastic mat, where large aggregates
could be broken apart gently, and fine roots (<2 mm) and adhering rhizosphere soil could be removed with forceps. Rhizosphere soil was operationally defined as soil adhering to roots after gentle shaking, while bulk soil was defined as not adhering soil [5]. Within several hours of collection, rhizosphere soil was carefully separated from fine roots using forceps and a brush. The percentage of rhizosphere soil mass in each composited sample was calculated on a mass basis (oven-dry weight) relative to the total mass of soil. All samples were temporarily kept in a cooler (5 °C), and they were transported to the lab within 12 h of collection from the field.

2.3. Laboratory Analyses

Fresh rhizosphere soil and bulk soil were passed through 2 mm sieve, and were used for all microbiological assays. Subsamples were dried to a constant mass at 105 °C to determine water content. The remaining soil was air-dried for analyzing soil chemical properties.

Potential soil C_{min} and net N_{min} were determined in an aerobic incubation experiment [12]. The incubation experiment for determining C_{min} and net N_{min} rates started within 2–3 days to minimize the effect of depletion of labile substrates during soil storage. Specifically, a thin layer of deionized water was spread on the bottom of a sealed plastic jar (500 mL). Two beakers (25 mL) were located in the jar side by side, with one filled with fresh soil (equal to 10 g of air-dried mass) and the other filled with 20 mL 1 mol/L NaOH solution. We placed these jars in an incubator (SHELLAB LI20-2, Sheldon Manufacturing Inc., Cornelius, OR, USA) at 25 °C for 30 days. The respired CO_{2}-C was trapped by the NaOH solution and measured by a Total Organic Carbon Analyzer (TOC-L CPH, Shimadzu, Kyoto, Japan) at the end of incubation. Meanwhile, the soil inorganic N (NH_{4}^{+}-N and NO_{3}^{-}-N) was extracted immediately after incubation. Then, the C_{min} and net N_{min} were calculated as cumulated CO_{2}-C and changed inorganic N (concentration at the end of incubation subtracts that at the start) divided by the days of incubation. The rates of C_{min} and net N_{min} were expressed by soil dry mass. We also calculated specific (per unit soil total C or total N) rates of C_{min}' and net N_{min}' to account for changes in soil total C or N between rhizosphere and bulk soils.

Microbial biomass C (MBC) was analyzed using the chloroform fumigation-extraction method [32]. After 24 h of fumigation, both fumigated and unfumigated soils were extracted with 40 mL 0.5 mol/L K_{2}SO_{4} (soil weight: solution volume = 1:4), and thus the extractable organic C was measured by a TOC Analyzer. The amount of MBC was calculated as the difference in extractable organic C between the fumigated and unfumigated soil samples and adjusted by a universal conversion factor of 0.45.

Soil extracellular enzymes such as β-glucosidase (BG), N-acetyl-β-glucosaminidase (NAG), and non-specific oxidative enzymes (POX, phenol oxidase; PER, peroxidase) were analyzed. The activities of these enzymes were determined using 96 well microplate fluorometric and spectrophotometric assays [33], with modification described in Jing et al. 2017. Briefly, we used sodium acetate buffer to make slurry (1.5 g fresh soil with 125 mL solution), adjusting pH to 4.5 in order to make it close to average pH across altitudes. All plates were incubated in the dark at 25 °C for 4 h for hydrolytic enzymes and for 24 h for oxidative enzymes. We used a microplate reader (BioTek Synergy 2, Winooski, VT, USA) to measure the fluorescence at 360 nm excitation and 460 nm emission for hydrolytic enzymes (BG and NAG) and absorbance at 450 nm for oxidative enzymes (POX and PER) [34]. Since the value of POX was relatively low and it always shared the same trend with PER, we present this as oxidase (OX).

Soil pH was determined in a 1:2 slurry (soil:deionized water) suspension with a pH meter (S210 SevenCompact™, Mettler, Germany). Soil total C and total N content were measured with an elemental analyzer (Elementar Vario EL III, Hanau, Germany). Soil NH_{4}^{+}-N and NO_{3}^{-}-N concentrations were analyzed colorimetrically on an autoanalyzer (AutoAnalyzer III, Bran + Luebbe GmbH, Germany) after the soil was extracted with 2 mol/L KCl solution (5 g fresh soil with 20 mL solution). Soil-extractable P were extracted from 5 g air-dried soil with 40 mL solutions (0.03 mol/L NH_{4}F and 0.025 mol/L HCl) after
shaking at 180 rpm for 0.5 h, the extractions were measured by an Atomic Absorption Spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan).

Soil-exchangeable cations (K, Ca, Na and Mg) were extracted with 1 mol/L NH₄OAc (2.5 g air-dried soil with 50 mL solution) at pH 7.0. Soil available trace elements (Fe and Mn) were extracted with 0.1 mol/L HCl (10 g air-dried soil with a 40 mL solution) at pH 7.0. After shaking at 180 rpm for 2 h, the extractions were filtered through Whatman qualitative filter paper and measured by inductively coupled plasma–mass Spectrometry (5100 ICP-OES, Agilent, NY, USA).

2.4. Calculations and Statistics

Relative rhizosphere effect (RE) was calculated as the percentage difference between paired rhizosphere and bulk soil samples for a given response variable using the following equation [5].

\[
RE(\%) = \frac{\text{Rhizosphere} - \text{Bulk}}{\text{Bulk}} \times 100\%
\]

To estimate the ecosystem consequences of rhizosphere effect, we multiplied the relative rhizosphere effects on C_{\text{min}} and net N_{\text{min}} by the percentage of rhizosphere soil mass (RSM) in the upper 10 cm of soil [5]. We estimated the ecosystem-level rhizosphere effect (RE_{eco}) on only the C_{min} and net N_{min} variables due to their important ecological relevance in ecosystem.

\[
RE_{eco}(\%) = RE \times RSM
\]

Four plots were treated as replication \((n = 4)\). Paired-samples \(t\)-test was used to compare the difference in measured soil variables between paired rhizosphere soil and bulk soil for each altitude (). We also used paired-samples \(t\)-test to analyze the REs on soil variables across altitudes \((n = 11)\). Univariate linear regression was used to examine the relationships between the RE and altitude, between RSM and altitude, and between RSM and soil water content. All the statistical analyses were considered as significant at the 0.05 level. In some instances, data were log transformed to normalize the distribution of residuals. All analyses were conducted using IBM SPSS Statistics 20.0 (IBM Corporation, Armonk, NY, USA).

3. Results

3.1. The Relative Rhizosphere Effects along the Altitudinal Gradient

All bulk soil properties (except for P and Mn) in the upper 10 cm of soil differed remarkably among altitudinal sites (Tables S1 and S2). Soil pH varied from 3.91 to 4.78 among eleven sites. Soil water content increased linearly with the increase in altitude \((R^2 = 0.48)\). Soil total C, ranged from 35.85 mg g⁻¹ in the 1300 m to 117.00 mg g⁻¹ in the 1700 m, but did not correlate with altitudes. Soil NH₄⁺—N, NO₃⁻—N, extractable P, K, Ca, Na, Mg, Fe, and Mn, on average, were 17.2, 3.9, 2.6, 14.1, 186.4, 3.4, 20.9, 3.7 and 1.1 mg kg⁻¹, respectively (Table S1). Soil MBC varied nearly 4 fold, and soil C_{min} and net N_{min} varied by more than 3 fold among eleven sites (Table S1).

The REs on C_{min} and net N_{min} were significantly positive across altitudes (Figure 1). The mean magnitudes of the REs on C_{min} and net N_{min} (unit, soil mass) were 122% and 188%, respectively. Moreover, the mean magnitudes of the REs on specific C_{min} and net N_{min} (unit, soil total C or total N) were 44% and 38%, respectively (Figure 1). Among the eleven sampling altitudes, the magnitudes of REs on C_{min} and net N_{min} varied from 46% to 267% and 39% to 189%, respectively (Figure 2). Furthermore, nine out of eleven altitudes showed significant positive REs on C_{min}. However, there was no clear altitudinal pattern of RE on C_{min} and net N_{min} (Figure 2).
Rhizosphere effect (% difference)

Figure 1. The means of rhizosphere effects on given variables, including soil carbon mineralization rate per gram soil ($C_{min}$), net nitrogen mineralization rate per gram soil ($N_{min}$), carbon mineralization rate per gram soil total carbon ($C'_{min}$), net nitrogen mineralization rate per soil total nitrogen ($N'_{min}$), microbial biomass carbon (MBC), β−glucosidase (BG), N−acetyl−β−glucosaminidase (NAG), oxidase (OX), pH, total carbon (TC), total nitrogen (TN), ammonium nitrogen (NH$_3^+$−N), nitrate nitrogen (NO$_3^−$−N), extractable phosphorous (P), extractable potassium kalium (K), extractable calcium (Ca), extractable natrium (Na), extractable magnesium (Mg), extractable ferrum (Fe), and manganese (Mn). Values are the means and error bars are the standard errors of the eleven altitudinal sites. Asterisks indicate significant differences between rhizosphere soil and bulk soil based on paired-samples $t$-tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure 2. Relationship of the rhizosphere effects on soil carbon mineralization rate per soil mass ($C_{min}$, (a)), carbon mineralization rate per soil total carbon ($C'_{min}$, (b)), nitrogen mineralization rate per soil mass ($N_{min}$, (c)), nitrogen mineralization rate per soil total nitrogen ($N'_{min}$, (d)) with elevation. There was no significant linear regression ($p > 0.05$). For each elevation, values are the means and error bars are the standard errors of replicated plots (n = 4). Asterisks indicate significant differences between rhizosphere soil and bulk soil based on paired−samples $t$-tests (* $p < 0.05$ and ** $p < 0.01$).
The REs on soil microbial biomass carbon (MBC) and two extracellular enzyme activities (BG and NAG) were significantly positive across altitudes (Figure 1). The REs on MBC, BG, and NAG, on average, were 74%, 36%, and 49%, respectively (Figure 1). Although the RE on oxidase (OX) was not significant across altitudes (Figure 1), two out of eleven altitudes of the RE on OX were significantly negative (Figure 3d). We did not found the significant linear regression relationships between REs and altitude (Figure 3).

![Figure 3. Relationship of the rhizosphere effects on microbial biomass carbon (MBC, (a)), β-glucosidase (BG, (b)), N-acetyl-β-glucosaminidase (NAG, (c)) and oxidase (OX, (d)) with elevation. There was no significant linear regression (p > 0.05). For each elevation, values are the means and error bars are the standard errors of replicated plots (n = 4). Asterisks indicate significant differences between rhizosphere soil and bulk soil based on paired-samples t-tests (* p < 0.05 and ** p < 0.01).](image)

The REs on soil TC, TN, NH$_4^+$—N, NO$_3^-$—N, P, K, Ca, Mg, Fe, and Mn were significantly positive across altitudes (Figure 1). The mean magnitudes of the REs on TC and TN were 59% and 49%, respectively. Moreover, the concentration of inorganic nitrogen and extractable P were higher in the rhizosphere soil than in the bulk soil (Figure 4). The mean magnitudes of REs on soil metal cations (K, Ca, Mg, Fe, and Mn) are generally varied within the range of 29%~56% (Figure 5). In contrast to the above soil properties, the RE on soil pH was significantly negative despite the small (~1.6%) intensity (Figure 1). Notably, the RE on extractable Na was not significant across altitudes, but significant positive effects were identified in three out of eleven altitudes (Figures 1 and 5). The linear regression relationships between REs on soil properties and altitude were not significant (Figures 4 and 5).
Figure 4. Relationship of the rhizosphere effects on soil pH value (pH, (a)), total carbon (TC, (b)), total nitrogen (TN, (c)), ammonium nitrogen (NH$_4^+$−N, (d)), nitrate nitrogen (NO$_3^-$−N, (e)) and extractable phosphorous (P, (f)) with elevation. There was no significant linear regression ($p > 0.05$). For each elevation, values are the means and error bars are the standard errors of replicated plots ($n = 4$). Asterisks indicate significant differences between rhizosphere soil and bulk soil based on paired−samples $t$-tests (* $p < 0.05$ and ** $p < 0.01$).
3.2. Ecosystem-Level Rhizosphere Effects on Soil C and N Mineralization

Based on the relative REs and the percentage of rhizosphere soil mass, the ecosystem-level REs were estimated. Across altitudes, the mean magnitude of ecosystem-level RE on C$_{\text{min}}$ was 19%, which ranged from 4% to 48% (Figure 6a). Similarly, the mean magnitude of ecosystem-level RE on net N$_{\text{min}}$ was 16%, which ranged from 3% to 34% (Figure 6b). Interestingly, we found that the intensity of ecosystem-level REs increased with the increase in altitude (Figure 6). It should be noted that the relative REs has no altitudinal pattern (Figure 2). Moreover, we found that the percentage of rhizosphere soil mass increased linearly with the increase in altitude (Figure 7a). Across altitudes, the percentage of rhizosphere soil mass was on average 17%, which ranged from 7% to 58%. The maximum value (58%) occurred at the highest altitude (2200 m), and dense root layers were found during field sampling in this tundra ecosystem. In addition, the percentage of rhizosphere soil mass increased with the increase in soil water content (Figure 7b).
found during field sampling in this tundra ecosystem. In addition, the percentage of rhizosphere soil mass increased with the increase in soil water content (Figure 7b).

**Figure 6.** Relationship of the rhizosphere effects on soil carbon mineralization rate ($C_{\text{min}}$, (a)) and soil nitrogen mineralization rate ($N_{\text{min}}$, (b)) with elevation ($n = 11$). The solid red lines represent linear regression, and the blue lines represent the 95% confidence intervals.

**Figure 7.** Relationships of the rhizosphere soil mass with elevation (a) and soil water content (b) ($n = 11$). The solid red lines represent linear regression, and the blue lines represent the 95% confidence intervals.

### 4. Discussion

#### 4.1. The Magnitude and Direction of Relative Rhizosphere Effects in Field Soils

Our results demonstrated that the REs on soil $C_{\text{min}}$ and net $N_{\text{min}}$ were positive across altitudes (Figure 1), and the magnitude of relative REs on soil $C_{\text{min}}$ and net $N_{\text{min}}$ varied from 39% to 267% among altitudes (Figure 2). These results suggest that there is a large variation in REs in the natural ecosystems. Notably, the magnitudes of RE calculated by per soil mass were greater than that by per soil total C and total N content (specific rhizosphere effects). This mainly result from the higher total C and total N contents in the rhizosphere soils. Our results are in line with previous studies, which commonly reported positive
REs [5,12]. Recently, a meta-analysis also reported that the average REs on C\textsubscript{min} and net N\textsubscript{min} of woody plants were 38% and 40%, respectively [10]. Given that the REs are likely regulated by many factors such as tree species, mycorrhizal types, and sites, the magnitudes of RE may vary greatly among individual studies [12,14,20]. In addition, evidence from potted plants relative to unplanted soils showed REs ranging from −50% to +380% with an average of 59% [6,9]. Overall, our results provided a set of new evidence for the REs on soil C and N mineralization in the natural ecosystems.

REs on soil microbial biomass and their enzyme activities were generally positive except for oxidase (Figure 1), suggesting that the presence of plant roots could promote microbial growth and activities. Plant living roots produce rhizodeposits and improve the physical and chemical properties of the surrounding soils, thereby providing food and microhabitats for microbial proliferation [1]. Simultaneously, microbes provide nutrients for plant growth by releasing extracellular enzymes to degrade soil organic matter [2]. This mutualistic relationships between living roots and microbes in the rhizosphere soils likely lead to positive REs on microbial properties [4,35]. Extensive studies reported that the microbial biomass and their activities were higher in the rhizosphere than in the bulk soil [12,13]. Notably, the intensity and direction of REs varied with the kinds of enzymes (Figures 1 and 3). For the oxidase, the negative and neutral REs were also commonly reported in the literature [14,22,36]. Oxidase is mainly for degrading resistant soil organic matter [37]. The low proportion of resistant soil organic matter in rhizosphere is a potential reason for explaining the neutral RE on oxidase activity [1]. Microbes produce a variety of extracellular enzymes, and each enzyme has substrate specificity [38]. Therefore, different enzymes may respond differently to the rhizosphere. Collectively, soil microbes and their activities were generally stimulated by the presence of living roots.

REs on most of the soil chemical properties were positive with exceptions to soil pH and Na (Figure 1). The REs on soil pH were inconsistent among altitudinal sites (Figure 4a). This result is not surprising because of positive, negative and neutral REs on soil pH reported in the previous studies [5,12,39]. Root exudates contain a substantial proportion of organic acids [1]. These organic acids can not only induce the decline in the soil pH in the rhizosphere, but also activate nutrients [40]. In addition, plant roots absorb soil nutrients through mass flow, resulting in nutrients accumulation in the rhizosphere [3]. Evidence from previous studies showed that soil total C, total N, available N, and available P accumulate in the rhizosphere, accompanied by higher microbial enzyme activities in the rhizosphere than in the bulk soil [16,41]. Compared with the number of studies on soil C and N, there are relatively few studies considering the REs on soil extractable cations and micronutrients [7]. Consistent with previous studies [17,39], we found that higher concentration of K, Ca, Mg, Fe, and Mn in the rhizosphere soil relative to the bulk soil (Figure 1). The RE on Na was not significant (Figure 1), probably because Na is easy to migrate in soil. At present, the mechanisms of these nutrients enrichment in rhizosphere are far from clear [7]. Our results suggest that plant living roots have significant effects on soil properties, and highlighted the need to explore the mechanism and regulatory factors of REs on soil nutrients in situ in the future.

4.2. Altitudinal Patterns of the Relative Rhizosphere Effects

There was no altitudinal pattern in REs on investigated soil assays (Figures 2–5). Two previous studies reported that the REs on soil C\textsubscript{min} (priming effects) decreased significantly with increased altitude in tropical and temperate forests [42,43]. However, our results were inconsistent with those of the previous studies. The two previous studies focused on general priming effects (C\textsubscript{min}) representing by the difference with and without external organic C addition [44]. The different representation methods of REs may cause the inconsistent results. The lack of altitudinal pattern of the REs could be potentially explained by the following reasons.

Firstly, in our incubation study with the uniform incubation temperature and soil moisture across the altitude gradient the influence of altitude on C\textsubscript{min} and net N\textsubscript{min} would
be weakened. Considering the great challenges of in situ measurements, the incubation method is a practical way at present. Several previous studies reported that the REs on $C_{\text{min}}$ were sensitive to the incubation temperature and soil moisture [45,46]. Secondly, the change of plant community composition may contribute to the altitudinal patterns. The magnitude and direction of REs varied greatly in different plant species [12,41]. Interestingly, the REs on $C_{\text{min}}$ and $N_{\text{min}}$ of the five elevations (1300, 1400, 1500, 1600, and 1700 m) belonging to dark coniferous forest showed approximate parabolic altitudinal patterns (Figure 2). However, there was no clear trend in REs on most of investigated soil assays along the four vegetation types (Figures S1–S4). Thirdly, altitude is a comprehensive factor including climatic factor, soil properties and vegetation types [23]. Our results showed that the REs on different variables were commonly correlated with each other (Figure S5), suggesting that the REs are mostly regulated by soil inherent properties. The altitude potentially regulates the REs by indirect pathways [43]. Collectively, there are many factors (e.g., climate, vegetation, soil properties, and microbial activity) co-varying with altitude, indicating the complexity of the altitudinal pattern of REs. Therefore, the relative contribution of these factors involving in the altitudinal pattern of REs call for further study.

4.3. The Estimates of Rhizosphere Effects on Soil C and N Mineralization at the Ecosystem Scale

Our estimates of REs on soil $C_{\text{min}}$ and net $N_{\text{min}}$ were 19.4% (range from 3.7% to 48.1%) and 15.5% (range from 3.0% to 33.9%) across altitudes at the ecosystem scale, respectively (Figure 6). A previous study reported that the ecosystem-level REs for $C_{\text{min}}$ and net $N_{\text{min}}$ were ranged from 1% to 15% in different monospecific tree species plantations [5]. Moreover, the ecosystem-level REs on $C_{\text{min}}$ and net $N_{\text{min}}$ were 21% and 18% in a deciduous hardwood forest, respectively [22]. Compared with these previous estimates, our results of natural forests are higher than those of tree plantations, but similar to those of hardwood forests. Therefore, we speculate that the interaction of multi-species roots in natural forests might enhance the REs. In addition, the results from the free-air CO$_2$ enrichment (FACE) experiment and modeling suggest that the intensity of REs may be up to 30%–50% at the ecosystem scale [6,8,47]. Given that the high intensity of REs on soil $C_{\text{min}}$ and net $N_{\text{min}}$, we believe that the REs do play a key role in governing soil C and N cycling in natural ecosystems.

Ecosystem-level REs on soil $C_{\text{min}}$ and net $N_{\text{min}}$ significantly increased with the increased altitudes (Figure 6). Furthermore, the ecosystem-level RE mainly depends on the proportion of rhizosphere soil mass, which increased from 7% at low altitude to 58% at high altitude (Figure 7a). Consistent with our results, several previous studies also reported that the percentages of rhizosphere soil mass (or rhizosphere soil volume) could vary greatly [5,8,22]. Actually, the percentage of rhizosphere soil mass is likely affected by many factors including soil moisture, soil texture, fine-root traits, and artificial sampling [5]. Our results confirm that the percentage of rhizosphere soil mass is positively correlated with soil water content (Figure 7b). Generally, the finer soil texture is considered to produce a higher percentage of rhizosphere soil [48]. However, the content of soil clay decreased with the increase in altitude in the north slope of Changbai Mountains [49]. Thus, soil texture is not the cause of the altitudinal trend of rhizosphere soil mass in the current study. The percentage of rhizosphere soil mass in alpine tundra was up to 58% (Figure 7a), suggesting that the high percentage may result from the high fine-root density of alpine tundra plants. Overall, our findings suggest that the percentage of rhizosphere soil mass is a key factor in evaluating the altitudinal pattern of ecosystem-level REs.

Studies on the ecosystem-level REs remain in its infancy stage because of the methodological challenge. In the current study, the percentage of rhizosphere soil mass was applied to scale up the REs to the ecosystem level [5]. Similarly, the ecosystem-level REs could also be estimated by the percentage of rhizosphere soil volume within a certain distance (e.g., 1 mm) from root surface [22]. The challenge of the above scaling methods is how to represent the range of rhizosphere robustly [8]. Although it is clear that the rhizosphere is a zone of soil surrounding living roots, the boundary is ambiguous ranging from sub-µm
to supra-cm scales [3]. Moreover, the zone of the rhizosphere is spatiotemporal dynamic since the growth of roots [48]. Nevertheless, the scaling methods based on the percentage of rhizosphere soil mass and volume are practicable to evaluate the ecosystem-level REs in the current stage [5,22]. Recently, a study reported that fine-root traits (e.g., root diameter and nitrogen concentration) are correlated with REs [19]. Developing the relationship between the REs and the stand indices (e.g., fine-root biomass) and representing the range of rhizosphere soil with fine-root traits, is an available way to examine the ecosystem-level REs in the future.

5. Conclusions

Knowledge on REs of mature trees is still limited, and this is rarely studied in natural forests. Our results revealed that plant living roots generally stimulate (positive REs) soil C and N processes, microbial activity, and soil extractable nutrients in the field ecosystems. These results suggest that the rhizosphere is a hotspot for soil C and nutrient biogeochemical cycles. Based on the relative REs and the percentage of rhizosphere soil mass approach, we roughly estimated the RE at the ecosystem level. The intensities of the ecosystem-level REs were large enough, which should be paid attention to when simulating soil C and N cycles. We also found that the ecosystem-level REs on C and N mineralization increased with the increased altitudes. These altitudinal patterns mainly result from the percentage of rhizosphere soil mass, which increased linearly with altitude. Therefore, reasonable identification of the rhizosphere soil range is very important to estimate ecosystem-level RE. In the future, we should explore the RE in more ecosystem types such as tropical forests. Moreover, the regulating factors and mechanism for the ecosystem-level RE deserve further study.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13071104/s1, Figure S1: The rhizosphere effects on soil processes of four vegetation types; Figure S2: The rhizosphere effects on microbial biomass carbon and enzymes activities of four vegetation types; Figure S3: The rhizosphere effects on soil basic properties of four vegetation types; Figure S4: The rhizosphere effects on soil extractable cations of four vegetation types; Figure S5: Pearson’s correlation matrix of rhizosphere effects on given variables across altitudinal sites; Table S1: Soil properties of bulk soil along the northern slope on Changbai Mountain, in northeast China; Table S2: The results of One-way ANOVA of bulk soil properties among eleven sites. The F values are shown in the table.

Author Contributions: C.H., P.W. and W.C. designed this study. C.H., J.L. and L.Y. performed the experiments. C.H., J.L., L.Y. and P.W. analyzed the data. C.H. wrote the first draft of the manuscript with contributions of all authors. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the National Natural Science Foundation of China (No. 31870429; 32071755; 31570620).

Data Availability Statement: The data are included in this article.

Acknowledgments: We thank Hongchao Zhu and Guanhua Dai for their assistance with sample collection and processing; Guilin Sun and Qiufeng Xu for their help with laboratory work. We are grateful to the Research Station of Changbai Mountain Forest Ecosystems for logistic support in the field.

Conflicts of Interest: The authors declare no conflict of interest.

References


26. Ren, C.; Zhou, Z.; Guo, Y.; Yang, G.; Zhao, F.; Wei, G.; Han, X.; Feng, L.; Feng, Y.; Ren, G. Contrasting Patterns of Microbial Community and Enzyme Activity between Rhizosphere and Bulk Soil along an Elevation Gradient. *Catenia* **2021**, *196*, 104921. [CrossRef]
