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The Contributions of Soil Fauna to the Accumulation of Humic Substances during Litter Humification in Cold Forests

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Abstract: Litter humification is an essential process of soil carbon sequestration in forest ecosystems, but the relationship between soil fauna and humic substances has not been well understood. Therefore, a field litterbag experiment with manipulation of soil fauna was carried out in different soil frozen seasons over one year in cold forests. The foliar litter of four dominated tree species was selected as Birch (*Betula albosinensis*), Fir (*Abies fargesii* var. *faxoniana*), Willow (*Salix paraplesia*), and Cypress (*Juniperus saltuaria*). We studied the contribution of soil fauna to the accumulation of humic substances (including humic acid and fulvic acid) and humification degree as litter humification proceeding. The results showed that soil fauna with litter property and environmental factor jointly determined the accumulation of humic substances (humic acid and fulvic acid) and humification degree of four litters. After one year of incubation, the contribution rates of soil fauna to the accumulation of humic substances were 109.06%, 71.48%, 11.22%, and −44.43% for the litter of fir, cypress, birch, and willow, respectively. Compared with other stages, both growing season and leaf falling stage could be favorable to the contributions of soil fauna to the accumulation of humic substances in the litter of birch, fir, and cypress rather than in willow litter. In contrast, the contribution rates of soil fauna to humification degree were −49.20%, −7.63%, −13.27%, and 12.66% for the litter of fir, cypress, birch, and willow, respectively. Statistical analysis indicated that temperature changes at different sampling stages and litter quality exhibited dominant roles in the contributions of soil fauna on the accumulation of humus and litter humifiaction degree in the cold forests. Overall, the present results highlight that soil fauna could play vital roles in the process of litter humification and those strengths varied among species and seasons.

Keywords: soil fauna; humic substances; humic acid; fulvic acid; humification degree

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1. Introduction

Humus has been known as the major component of soil organic carbon, which can improve soil fertility and soil physical and chemical structure [1–3]. Litter humification is an important pathway for the formation of soil humus and plays an important role in maintaining soil fertility and carbon sequestration in nature ecosystems [4–7]. Soil fauna are not only decomposers during litter humification, but also act as boosters throughout the formation of soil organic matter by producing and transferring humic substances [8–11]. The roles of soil fauna on the process of decomposition have been wildly documented, and the effect of soil fauna on litter decomposition can be elucidated from different functional groups and feeding habits [12–14]. However, their impacts on litter humification have not been well understood. Therefore, there is still a lack of unified cognition on the role of soil fauna in the accumulation of humus during litter humification.

Generally, the litter humification by soil fauna works through nesting, constructing shuttle, foraging fragmentation, and regulating microorganism's structure [15]. Additionally, these feces and dead bodies of soil fauna are the main sources of soil humus [16,17]. Moreover, some species of soil fauna can weaken microbial activities and feed humic substances, displaying negative effects on the accumulation of humic substances [18]. Including other factors, microclimate and litter quality are widely considered as vital constraints regulating the relationship between soil fauna and litter humification [19–21]. Humic substances are more sensitive and unstable in cold forests than those are in subtropic/tropical forests, while stable temperature and humidity are more likely to promote the formation of humic substances [22–24]. The feeding of soil fauna may be limited under low temperature conditions, thereby affecting the formation of humic substances [25]. Nevertheless, litter quality and its interaction with living conditions of soil fauna could control the humus accumulation by regulating the community structure of soil fauna [22], leading to currently inconsistent online information.

The cold forest on the eastern edge of the Qinghai–Tibet Plateau is an important part of the ecological barrier in the upper reaches of the Yangtze River [26]. The forests have served as prominent strategic positions in regional climate regulation, biodiversity conservation, water conservation, and river runoff regulation [27,28]. However, the cold forests are also fragile and irreversible, since they could be affected by geology and long-term snow cover and seasonal freeze–thaw cycles [29–31]. As an important indicator of soil fertility, soil humus is one of the essential factors in keeping forest soil productivity [32,33]. However, the processes of litter humification and its relationships with soil fauna have not been well addressed in the cold regions. Therefore, a field incubation has carried out to investigate the contributions of soil fauna in the accumulation of humus for the foliar litter of four dominated tree species. The following hypothesis is proposed: (1) soil fauna could promote the process of humidification depending on species and key times in this region; (2) the individual density of soil fauna is related to the degree of humification of leaf litter.

2. Materials and Methods

2.1. Site Description

The study site was conducted at the Long-term Research Station of Alpine Forest Ecosystem of Sichuan Agricultural University (31°14′–31°19′ N, 102°53′–102°57′ E, 2458–4619 m a.s.l), which is located in the eastern Tibet Plateau [34,35]. The average annual temperature is 2 °C, and the average annual rainfall in this site is 850 mm. The seasonal freeze–thaw period is from November to April and lasts for 5 to 6 months each year. The experimental sites are located at two elevations, where the higher elevation (3593 m) is a primary natural forest that is dominated with Willow (*Salix paraplesia* Schneid) and Cypress (*Juniperus saltuaria* (Rehd. et Wils) Cheng et W. T. Wang), whereas the other site (3028 m) is a plantation that dominated with Birch (*Betula albosinensis* Burkill) and Fir (*Abies fargesii* var. *faxoniana* (Rehder & E. H. Wilson) Tang S. Liu) trees. The soil is a Cambic Umbrisol (IUSS Working Group WRB, 2006), and more information on soil properties can be found in Tan et al. (2020) [36].

2.2. Litterbag Experiment and Sample Collection

A primary natural forest and a cypress plantation were selected in the study site. Fresh senesced foliar litters were collected through a 5 m × 5 m litter collector at each site in October 2013. All litter materials were air-dried at room temperature (25 °C) for 15 days and then weighted 10 ± 0.05 g for each sample that was placed in a 20 cm × 20 cm litterbag with one type of foliar litter. Litterbags with two mesh sizes were used to exclude and permit the access of soil fauna into the litterbags to determine fauna effects on litter humification [37]. Specifically, there were two types of litterbags, with both types having the bottoms of the litterbags with a mesh size of 0.04 mm, but the tops had two mesh sizes (0.04 mm and 3.00 mm): 0.04 mm for the treatment to exclude the entry of soil fauna (fauna exclusion) and 3.00 mm for the control to permit the entry of macro-, meso- and microfauna [36,38]. A total of 360 litterbags (2 meshes × 4 species × 5 times × 3 replicates × 3 plots) were randomly distributed on the soil surface (with a distance between litter bags of about 5 cm) just after the litterfall peak in November 2013.

The temperature of litterbag was measured using DS1923-F5 Recorders (iButton DS1923-F5, Maxim/Dallas Semiconductor, Sunnyvale, CA, USA) every 2 h at each site and used to calculate the daily average (DAT) and total accumulated temperature (TAT, the accumulation of daily average temperature in the litterbag during each stage) during the whole experimental periods. The average daily temperature in the alpine forests was showed in Figure 1. The trend of the daily average temperature of the primary forest and the plantation forest is basically the same, but from the growing season to the leaf falling stage period, the daily average temperature of the plantation forest is higher than that of the primary forest. The water content of litter was dried into oven for 48 h until its weight was constant, then it was recorded.

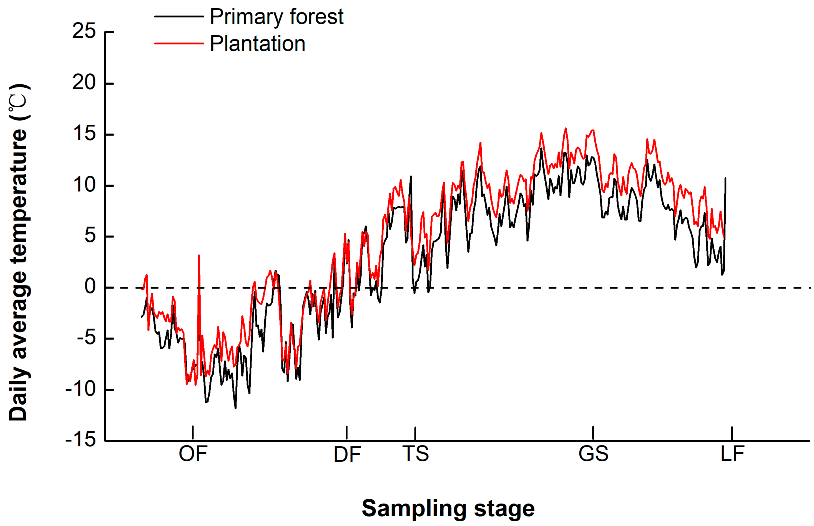

Figure 1. The average daily temperature in the alpine forests of different sampling stages. OF: onset of freezing, DF: deep freezing stage, TS: thawing stage, GS: growing season, LF: leaf falling stage.

According to our previous research and long-term sequential temperature observations [39], litterbags were collected in different stages: onset of freezing stage (OF, December 2013), deep freezing stage (DF, March 2014), thawing stage (TS, April 2014), growing season (GS, August 2014), and leaf falling stage (LS, October 2014). Thereafter, 9 litterbags filled with each litter species were randomly collected at each sampling site at every stage. A part of the recovered litterbags was used for soil fauna collection and the determination of matrix quality. The other part was air-dried to remove impurities, then oven-dried at 65 °C for 48 h (DHJ 2450B, Sheng Yuan Instrument, Zhengzhou, China) and weighed (Figure 2). The air-dried fallen leaves were crushed, sieved (0.25 mm mesh), and stored in a kraft paper bag for the extraction experiment of humus.

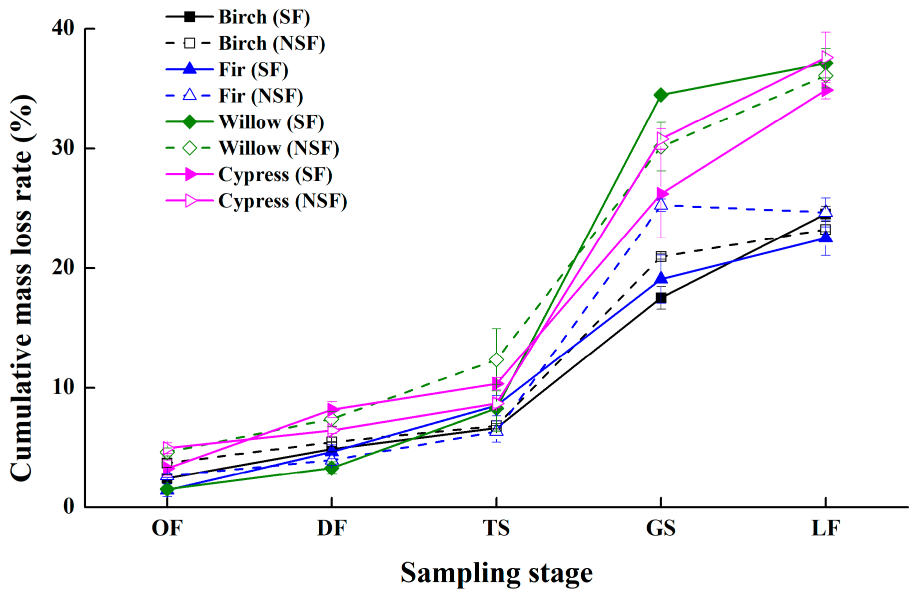

Figure 2. Cumulative mass loss rates of four litter (excluded (NSF) and non-excluded soil fauna (SF)) at different sampling stages (mean ± standard error, n = 3). OF: onset of freezing, DF: deep freezing stage, TS: thawing stage, GS: growing season, LF: leaf falling stage.

2.3. Soil Fauna Collection and Chemistry Analysis

The soil fauna in the litterbags were extracted by the Tullgren funnel method over a period of 48 h at 30 °C, as previously described [37]. All extracted soil fauna were counted and classified under a microscope (Leica MZ 125, Leica Microsystems GmbH, Wetzlar, Germany). After one year of exposure, we collected a total number of 2005 soil fauna individuals in the litter bag, among which Collembola (*Isotomidae* and *Campodeoidea*, etc.) and mites (*Oribatida* and *Prostigmata*, etc.) were the dominant groups, accounting for 49% and 46% of the total number of soil fauna, respectively (Figure 3, Supplementary Materials Table S1).

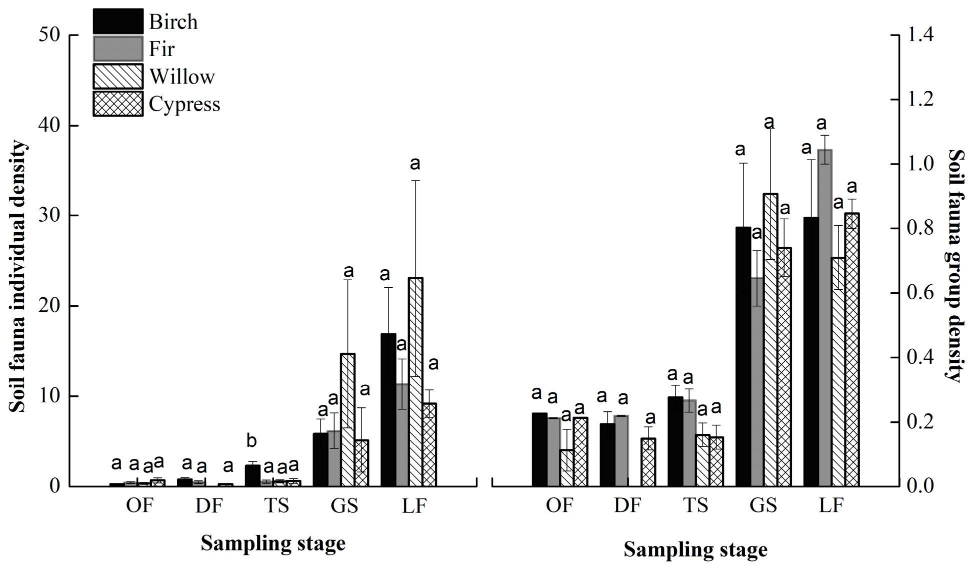

Figure 3. Individual and group density of soil fauna in different sampling stage (mean ± standard error, n = 3). OF: onset of freezing, DF: deep freezing stage, TS: thawing stage, GS: growing season, LF: leaf falling stage. The letters a and b mean in the sampling stage with different letters differ significantly, $p < 0.05$.

NaOH method was used to extracted humic substances [40]. 0.5 g of litter sample was added to 100 mL of 0.1 mol/L NaOH + $Na_4P_2O_7 \cdot 10\ H_2O$ mixed solutions, shaken for 10 min, and put in a water bath with a constant temperature at 100 °C for 1 h. The liquid was filtered and used in part to measure humic substances (HS). We took out another 20 mL, added 0.5 mol/L H_2SO_4 to pH 3, and put it in a water bath with a constant temperature at 80 °C for 30 min. The liquid was taken out and filtered, and then the precipitated part was dissolved with 0.05 mol/L NaOH solution for the determination of humic acid (HA). The humic substances and the humic acid test solution were both filtered with a 0.45 µm filter membrane, and then measured with TOC analyzer (multi N/C 2100, Analytik Jena, Thüringen, Germany).

2.4. Data Calculation and Statistical Analyses

Soil fauna individual density (D_i) and group density (D_g) were calculated as [37]:

$$\text{Individual density } (D_i) = N_i/M$$

$$\text{Group density } (D_g) = N_g/M$$

where, N_i, N_g, and M represent the individual number of soil fauna, the group number of soil fauna, and the mass residue of litter, respectively.

Fulvic acid concentration (FA), humification degree (HD), the contribution rate of soil fauna to the accumulation of humic substances, humic and fulvic acid, and humification degree were calculated as [30,36]:

$$\text{FA (g/Kg)} = \text{HS} - \text{HA}$$

$$\text{HD (\%)} = \text{HS/OC} \times 100\%$$

$$C_{HS}\ (\%) = (HS_{(SFt)} \times M_{(SFt)} - HS_{(NSFt)} \times M_{(NSFt)})/HS_{(NSFt)} \times M_{(NSFt)} \times 100\%$$

$$C_{HA}\ (\%) = (HA_{(SFt)} \times M_{(SFt)} - HA_{(NSFt)} \times M_{(NSFt)})/HA_{(NSFt)} \times M_{(NSFt)} \times 100\%$$

$$C_{FA}\ (\%) = (FA_{(SFt)} \times M_{(SFt)} - FA_{(NSFt)} \times M_{(NSFt)})/FA_{(NSFt)} \times M_{(NSFt)} \times 100\%$$

$$C_{HD}\ (\%) = (HD_{(SFt)} \times M_{(SFt)} - HD_{(NSFt)} \times M_{(NSFt)})/HD_{(NSFt)} \times M_{(NSFt)}$$

where HS and HA represent the concentrations of humic substances and humic acid, OC represents organic carbon concentration of litter. C_{HS}, C_{HA}, C_{FA}, and C_{HD} represent the contribution rate of soil fauna to the accumulation of humic substances, humic and fulvic acid, and humification degree, respectively. $HS_{(SFt)}$, $HA_{(SFt)}$, $FA_{(SFt)}$, and $HD_{(SFt)}$ represent the accumulation of humic substances, humic and fulvic acid, and humification degree under non-excluded soil fauna at the stage t, respectively. $HS_{(NSFt)}$, $HA_{(NSFt)}$, $FA_{(NSFt)}$, and $HD_{(NSFt)}$ represent the accumulation of humic substances, humic and fulvic acid, and humification degree under excluded soil fauna at the stage t, respectively. $M_{(SFt)}$ and $M_{(NSFt)}$ represent the mass residue of excluded soil fauna and non-excluded soil fauna at the stage t.

One-way ANOVA was used to analyze individual density and group density of soil fauna and the contribution rate of soil fauna to humic substances, humic acid, fulvic acid, and humification degree accumulation. Multivariate analysis of variance was used to analyze the individual density and group density of soil fauna, accumulation of humic substances, humic acid, fulvic acid, and degree of humification with species time, and their interaction. Pearson correlation analysis was used to calculate the correlation parameters of the contribution rate of soil fauna to the accumulation of humic substances (HS), humic acid (HA), fulvic acid (FA), and humification degree (HD) to carbon, nitrogen, phosphorus, lignin (L), cellulose (CE), total accumulated temperature (TAT), daily average temperature (DAT), litter water content (LWC), soil fauna individual density (D_i), and group density (D_g) at different stages. Linear regression analysis was used for the individual and group density of soil fauna and the contribution rate of soil fauna to the degree of litter humification. All significant differences were set at a level of $p = 0.05$. All statistical analyses were performed with SPSS 20.0 (IBM SPSS Statistics Inc., Chicago, IL, USA).

3. Results

3.1. The Contributions of Soil Fauna on Humic Substances Accumulation

Soil fauna showed significant effects on the accumulation of humic substance, but these strengths differed among sampling time and species (Figure 4 and Table 1). Over one year incubation, the contribution rates of soil fauna to humic substances were 109.06%, 71.48%, 11.22%, and −44.43% for fir, cypress, birch, and willow, respectively. Pearson correlation showed that soil fauna group density was significantly correlated with the contribution rates of soil fauna to humic substances at OF stage ($r = -0.59$, $p < 0.05$, Table 2). At the stage of OF, soil fauna in willow litter promoted the accumulation of humic substances, while it inhibited the accumulation of humic substances in fir and birch litters. Moreover, soil fauna improved the accumulation of humic substances at both the GS and LF stages in fir, birch, and cypress litters, but negative effects of soil fauna on the accumulation of humic substances in willow were observed during both stages.

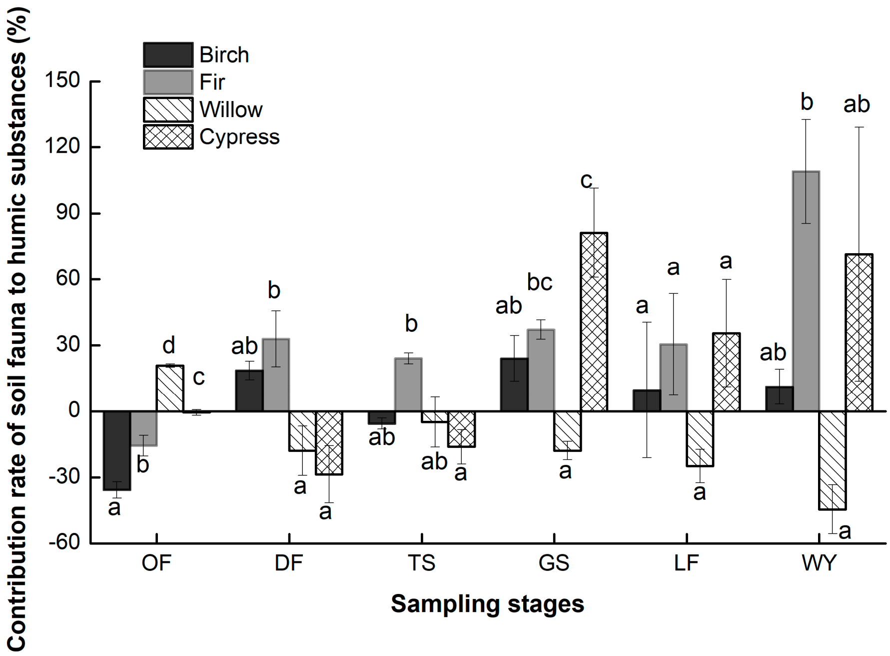

Figure 4. The contribution rate of soil fauna to the accumulation of four litter humic substance in different stages (mean ± standard error, n = 3). OF: onset of freezing stage, DF: deep freezing stage, TS: thawing stage, GS: growing season, LF: leaf falling stage, WY: whole year. The letters a–c mean in the sampling stage with different letters differ significantly, $p < 0.05$.

Table 1. *F* values for multivariate analysis of variance for individual (D_i) and group density (D_g) of soil fauna, the contribution rate of humic substances (HS), humic acid (HA), fulvic acid (FA), and humification degree (HD) by species and time.

Factor	D_i	D_g	HS	HA	FA	HD
Species (S)	2.04	0.67	5.38 **	23.79 ***	73.71 ***	2.470
Time (T)	12.23 ***	54.46 ***	5.43 **	9.63 ***	35.88 ***	10.04 ***
S × T	0.73	1.13	4.75 ***	26.73 ***	36.65 ***	8.09 ***

** $p < 0.01$. *** $p < 0.001$.

Table 2. Pearson correlation analysis of contribution rate of soil fauna to humic substances (HS), humic acid (HA), fulvic acid (FA) and humification degree (HD) and C, N, P, lignin (L), cellulose (CE), total accumulated temperature (TAT), daily average temperature (DAT), litter water content (LWC), soil fauna individual density (D_i), and group density (D_g) at different stages. OF: onset of freezing stage, DF: deep freezing stage, TS: thawing stage, GS: growing season, LF: leaf falling stage.

Time	Factor	C	N	P	L	CE	TAT	DAT	LWC	D_i	D_g
OF	HS	−0.72 **	0.64 *	0.77 **	−0.52	0.68 *	−0.85 **	−0.85 **	−0.47	0.173	−0.59 *
	HA	−0.42	0.72 **	0.67 *	−0.22	0.63 *	−0.48	−0.48	−0.37	−0.33	−0.39
	FA	−0.69 *	0.62 *	0.76 **	−0.55	0.69 *	−0.86 **	−0.86 **	−0.45	0.21	−0.52
	HD	−0.32	0.63 *	0.14	0.02	0.06	−0.04	−0.04	−0.56	−0.33	−0.47
DF	HS	−0.46	−0.16	−0.63 *	0.43	−0.66 *	0.83 **	0.83 **	−0.51	0.51	0.49
	HA	−0.33	−0.57	−0.74 **	0.59 *	−0.75 **	0.89 **	0.89 **	0.08	0.45	0.48
	FA	−0.54	−0.14	−0.69 *	0.37	−0.71 *	0.82 **	0.82 **	−0.55	0.59	0.43
	HD	−0.29	0.75 **	0.04	−0.07	0.01	−0.17	−0.17	−0.31	0.24	0.35
TS	HS	0.41	−0.02	−0.26	0.09	0.29	0.54	0.54	−0.64 *	−0.17	0.44
	HA	0.46	−0.21	−0.53	0.25	0.34	0.76 **	0.76 **	−0.86 **	−0.01	0.69 *
	FA	−0.02	0.32	0.11	−0.4	0.03	0.17	0.17	−0.41	−0.51	0.08
	HD	−0.13	−0.12	0.05	0.35	−0.23	−0.28	−0.28	0.49	0.61 *	−0.11
GS	HS	−0.21	−0.77 **	0.72 **	−0.47	0.34	−0.01	−0.01	−0.38	−0.35	−0.33
	HA	0.32	−0.26	0.01	0.57	−0.62 *	0.39	0.39	0.54	−0.16	−0.01
	FA	−0.48	−0.37	0.33	−0.71 **	0.61 *	−0.21	−0.21	−0.59 *	−0.18	−0.34
	HD	0.02	0.23	−0.01	−0.13	−0.24	−0.44	−0.44	−0.23	0.14	0.32
LF	HS	−0.03	−0.56	−0.33	−0.12	0.54	0.18	0.18	−0.49	−0.49	0.13
	HA	0.56	−0.16	0.21	0.02	0.51	−0.04	−0.04	−0.59 *	−0.28	0.42
	FA	−0.2	−0.71 **	−0.48	0.44	0.60 *	0.75 **	0.75 **	−0.34	−0.31	0.62 *
	HD	0.05	−0.67 *	−0.45	−0.55	0.63 *	−0.08	−0.08	−0.65 *	−0.23	0.02

* $p < 0.05$; ** $p < 0.01$.

3.2. The Contributions of Soil Fauna to Humic Acid Accumulation

The contribution of soil fauna to accumulation of humic acid was significantly affected by species (F = 23.79), sampling time (F = 9.63), and their interaction (F = 27.53, all $p < 0.001$, Table 1). Overall, the contribution rate of soil fauna to the accumulation of humic acid showed the order as fir (111.87%) > birch (91.24%) > cypress (−34.83%) > willow (−49.9%). Soil fauna group density was significantly correlated with the contribution rate of soil fauna to humic acid at TS stage (r = 0.69, $p < 0.05$, Table 2). Significant increases in the accumulation of humic acid contributed by soil fauna were detected in willow litter rather than in other species at OF stage (Figure 5). In contrast, the contributions of soil fauna to humic acid exhibited the same trend with that soil fauna promoted the accumulation of humic acid in the litters of birch and fir but inhibited them in the litters of willow and cypress at DF and TS stages. Soil fauna promoted the accumulation of humic acid in birch litter at GS and LF stages, while limiting the accumulation in other litters.

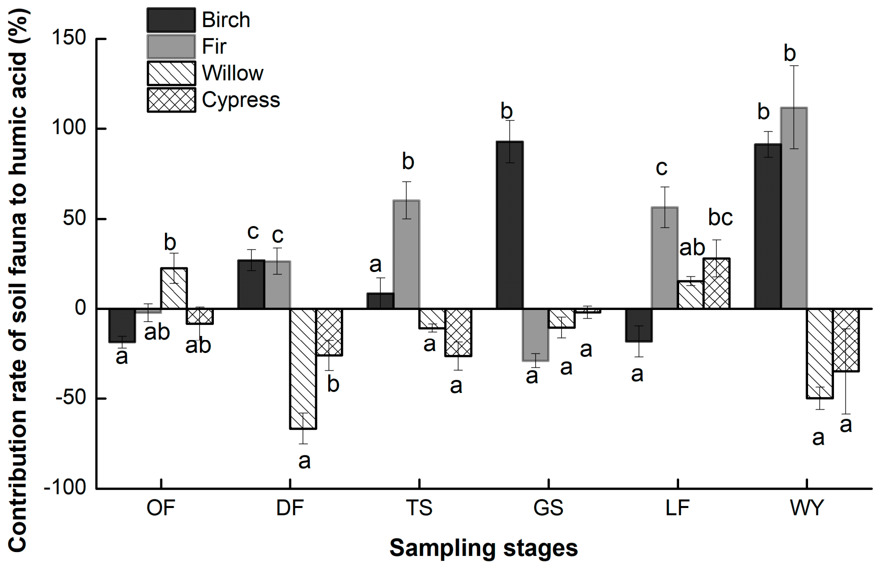

Figure 5. The contribution rate of soil fauna to the accumulation of four litter humic acid in different stages (mean ± standard error, n = 3). OF: onset of freezing stage, DF: deep freezing stage, TS: thawing stage, GS: growing season, LF: leaf falling stage, WY: whole year. The letters a–c mean in the sampling stage with different letters differ significantly, $p < 0.05$.

3.3. The Contributions of Soil Fauna to Fulvic Acid Accumulation

The contribution rate of soil fauna to the accumulation of fulvic acid showed the order as, fir (220.24%) > cypress (35.43%) > willow (−28.24%) > birch (−55.64%). Soil fauna promoted the accumulation of fulvic acid in the litter of willow and cypress but limited the accumulations of fulvic acid in other two litters at OF stage (Figure 6), which showed the opposite side at OF stage. Soil fauna promoted the accumulation of fulvic acid in the litter of fir and willow and inhibited the accumulation in the litter of birch and cypress at TS stage. At the stage of GS, soil fauna promoted the accumulation of fulvic acid in the litters of fir and cypress but inhibited the accumulation in the litters of birch and willow. Soil fauna only inhibited the accumulation of fulvic acid in willow litter at LF stage, whereas soil fauna contributed 40.42% and 128.10% increase of fulvic acid in the litters of birch and fir, respectively.

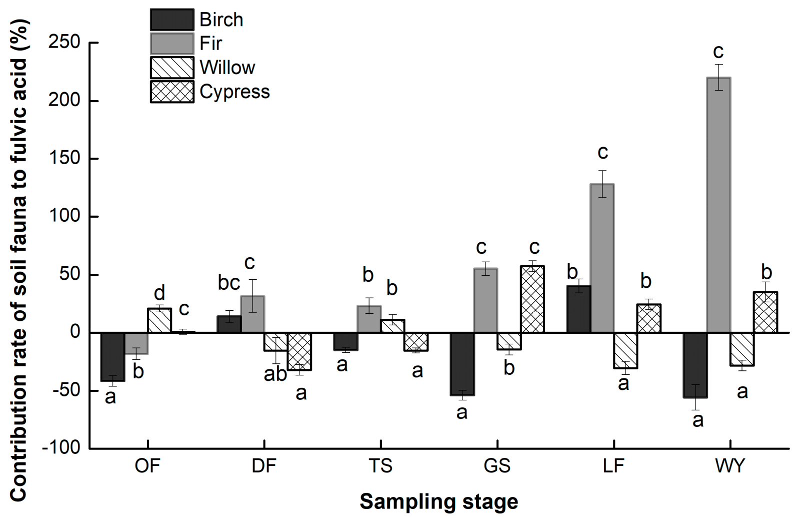

Figure 6. The contribution rate of soil fauna to the accumulation of four litter fulvic acid in different stages (mean ± standard error, n = 3). OF: onset of freezing stage, DF: deep freezing stage, TS: thawing stage, GS: growing season, LF: leaf falling stage, WY: whole year. The letters a–d mean in the sampling stage with different letters differ significantly, $p < 0.05$.

3.4. Contributions of Soil Fauna to Litter Humification Degree

Sampling time and the interaction of species with sampling time significantly affected the contribution rate of soil fauna on litter humification degree (F = 10.04 and F = 8.09, all p < 0.001, Table 1). The whole year contribution rate of soil fauna to humification degree was 12.66% for willow litter, and positive contribution appeared at both OF and DF stages (Figure 7). Contrarily, soil fauna showed −13.27%, −49.20%, and −7.63% of contribution rates to humification degree in the litter of birch, fir, and cypress in the whole year. However, the contribution rates of soil fauna to humification degree were 14.67%, 6.55%, and 48.79% in the litter of birch, fir, and cypress at LF stage.

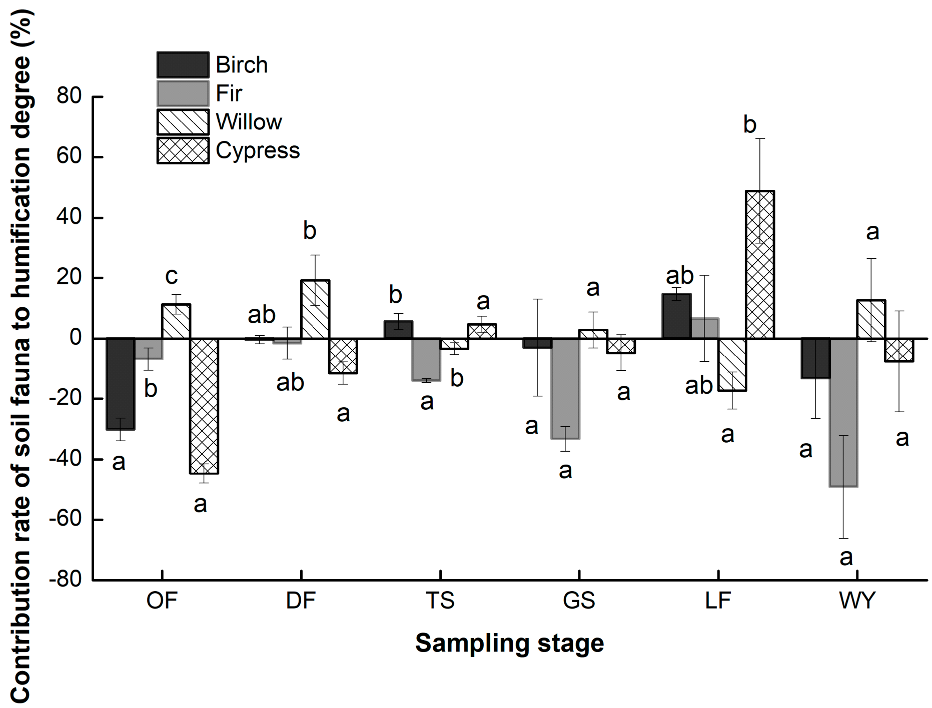

Figure 7. The contribution rate of soil fauna to four litter humification degree in different stages (mean ± standard error, n = 3). OF: onset of freezing stage, DF: deep freezing stage, TS: thawing stage, GS: growing season, LF: leave falling stage. OF: onset of freezing stage, DF: deep freezing stage, TS: thawing stage, GS: growing season, LF: leaf falling stage, WY: whole year. The letters a–c mean in the sampling stage with different letters differ significantly, p < 0.05.

The linear regressions show the correlation of individual and group density of soil fauna with cumulative contribution rate of soil fauna to the litter humification degree differed among species (Figure 8). Soil fauna significantly influenced the humification degree of the litter of birch, willow, and cypress, while it had no significant effect for fir litter. There was a significant correlation of individual density of soil fauna with humification degree for the litter of birch (p < 0.05, R^2 = 0.27, Figure 8a) and cypress (p < 0.01, R^2 = 0.37, Figure 8d). Additionally, there were significant correlations of group density for the litter of willow (p < 0.05, R^2 = 0.28, Figure 8c) and cypress (p < 0.05, R^2 = 0.28, Figure 7d).

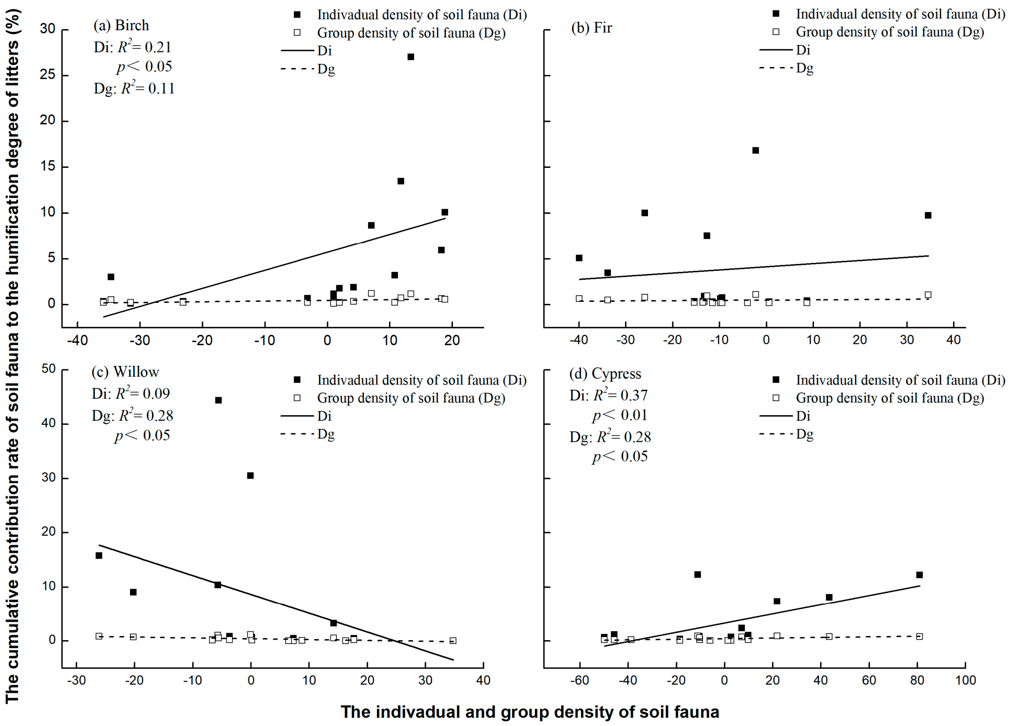

Figure 8. Linear regression analysis between the cumulative contribution rate of soil fauna to the humification degree of four kinds of litters and the individual and group density of soil fauna.

4. Discussion

Following our first hypothesis, we verified that the process of humidification promoted by soil fauna differed on species. Except for willow, we found soil fauna significantly promoted the accumulation of humic substances in litters of birch, fir, and cypress (Figure 4), indicating soil fauna could promote the process of humidification depending on species [37,41]. Additionally, we found soil fauna individual density significantly related to humification degree besides cypress (Figure 8), possibly by the presence of high concentration of volatile organic compounds in cypress, which could inhibit the activity of soil decomposer [42]. Overall, our result clearly demonstrated that soil fauna promotes the process of litter humification but varied by species and environmental factor.

Species, sampling, stage and their interactions had significant effects on the humic substances accumulations as litter humification proceeding in the alpine forests (all $p < 0.001$, Table 1). The positive effect of soil fauna on the accumulation of humic substances is high in the stages of GS and LF, which is in accordance with the densities of individual and group of soil fauna, indicating that soil fauna is favorable to the accumulations of humic substance of birch, fir, and cypress. Our result showed that soil fauna plays vital roles during the period of accumulation of humic substances, agreement with previous studies [19,43]. However, soil fauna (*Prostigmata* and *Oribatida*, etc.) also feed on humic substances and reduce the content of humic acid through the intestines, thereby converting organic matter [16], but these activities are varied among seasons and resulting in the capability of producing humic substances are difference. Interestingly, soil fauna had a positive effect on the accumulation of humic substance of willow litter in each stage aside from the stage of OF, while inhabited other foliar litters (Figure 4). The presence of scavenging soil fauna was found in birch, fir, and cypress litter, but not in willow litter (Supplementary Materials Table S1), so the humic substances might be highly consumed and decomposed by both soil microbes and scavenging fauna [8,10]. Moreover, we found the overall contribution rate of soil fauna to humic substance in coniferous litters (fir and cypress) is higher than that in broadleaf litters (birch and willow), which is related to litter quality. As higher contents of lignin in coniferous litters are more conducive to the formation of humus than broadleaf litters [43,44].

The contribution rate of litter humic substance response to soil fauna differs among sampling time, which is affected by litter qualities and environmental factors (Table 2). For fir litter, soil fauna had positive effects on both humic acid and fulvic acid, indicating a net accumulation of humic substances on the whole year, which was influenced by litter substrate quality [43]. Moreover, temperature and humidity can affect the biodiversity of soil fauna [44], and the growing season increases with soil fauna density and individual density, resulting in the contribution rate of soil fauna on fulvic acid being much higher than humic acid, as the contribution of soil fauna to humic acid was negative in the stage of GS (Figure 5). More importantly, after one-year incubation, the contribution rate of litter humic acid to soil fauna seems related to elevation, as positive contribution of birch and fir litters appeared in the plantation and negative values of willow and cypress litter in the primary forest. Differently, the contribution rate of litter fulvic acid to soil fauna was related to species, which showed negative contributions in coniferous litters and positive values in broadleaf litters. The divergent responses of humic acid and fulvic acid to soil fauna in different litter species highlighted that environmental factors and litter quality jointly controlled the content of humic substances [4,20,44].

Generally, environment exerts significant effects in the early decomposition stages due to physical effects [39]. When litter components that are susceptible to physical effects are consumed, the influence of the environment on litter decay is weakened. Otherwise, environmental factor directly affected the individual density and group density of soil fauna in each stage (Table 2), thus affecting the process of litter humification. We found the contribution rates of humic acid and fulvic acid to soil fauna reached maximum values in litters of birch, fir, and cypress at the stages of GS and LF. The individual density and group density of soil fauna both reached their peak values under the influence of temperature in these two stages, so their contribution rate reached the maximum. Moreover, Larionova et al. (2017) found there was an optimal temperature (12 °C) for the formation of humic substances [24]. In our site, the mean air temperature is 11 °C in the growing season, which provides good conditions to promote the humic substances of the fallen leaves accumulate rapidly. The annual contribution rate of soil fauna to the accumulation of humic acid in the plantation forest is higher than that of primary forest, and the contribution rate of the fulvic acid accumulation in coniferous litter is higher than that in broad-leaved litter, which indicates that the formation of humic acid by soil fauna is mainly affected by environmental factors, while the formation of fulvic acid is mainly affected by the quality of the matrix.

Over one-year field incubation, only willow litter showed a positive effect on the humification degree by soil fauna, and other litters were negative values (Figure 6). The different responses of humification degree to soil fauna in litters appeared significantly in the stages of OF and DF (Figure 7). The positive effects in willow litter at the stages of OF (16%) and DF (22%) indicate that soil fauna also play an important function, improving humification degrees even in cold winter. Previous studies have suggested a nonnegligible process of litter humification in winter, but their humification degree depends on litter quality [4]. Otherwise, the humification degree is positively correlated with the individual and group density of soil fauna (Figure 8), but this relationship is also controlled by litter quality and type [8,20]. We found the individual density of soil fauna in the litter of birch and cypress significantly correlated with humification degree, and the group density for willow and cypress litters was significantly correlated with the humification degree. In contrast, the fir litter showed no significant effect to litter humification mediated by soil fauna.

For litters of birch, fir and cypress, the annual contributions of soil fauna to the humification degree are negative, ranging from −48% to −8% after a year field incubation (Figure 7), indicating soil fauna may not be a key factor controlling the humification degree of these litters; other factors, such as initial litter quality and environmental factory, are also important [8,11,45]. The contribution rate of soil fauna to humification degrees reached the maximum values in the stage of LF (Figure 7), which are in accordance with the patterns of individual density and group density (Figure 3 and Supplementary Materials Table S1). Other studies also showed that the population of soil fauna has a positive correlation with the humification degree of litter [4,45].

5. Conclusions

The accumulation of humic substances (including humic acid and fulvic acid) and litter humification degree were jointly determined by soil fauna, species, and environmental factors. Soil fauna is favorable to the accumulation of humic substances in growing and leaf falling seasons for the litter of birch, fir, and cypress rather than willow litter. Moreover, the accumulations of humic acid and fulvic acid were promoted by soil fauna in fir litter, but these accumulations were inhibited in willow litter. Additionally, soil fauna showed positive effects on the humification degree in willow litter, but negative effects on the other three litter. Overall, our study gives a deep insight in the contribution of soil fauna on litter humification, which could provide useful data in soil carbon sequestration in the cold forests.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13081235/s1, Table S1: The average densities and eating patterns of soil fauna in the four studied litters under different stages.

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