Postprint: Effects of Soil Fauna at Different Elevations on Winter Litter Humification in Alpine Ecosystems of Western Sichuan

Authors: Tan Yu, Yang Wanqin, Liao Shu, Peng Yan, Li Jun, Wu Fuzhong

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Abstract

The humification process of litter during winter represents a critical stage for soil organic matter formation and carbon sequestration in alpine ecosystems, and may be influenced by soil fauna that maintain certain activity even under harsh freezing conditions, yet this has received insufficient attention. Therefore, using typical litter from alpine forests and meadows at elevations of 3000, 3600, and 4000 m in the alpine-gorge region of western Sichuan as study materials, and employing litterbags with different mesh sizes to exclude soil fauna, we investigated the effects of soil fauna on the litter humification process during different freeze-thaw periods (pre-freezing period, deep freezing period, and thawing period) from November 2013 to April 2014, following the natural decomposition process of litter. The results demonstrated that, based on analysis of the color coefficient (ΔlogK) and optical density values (E4>E6), soil fauna promoted litter humification as temperature decreased, while inhibiting litter humification as temperature increased during winter in alpine ecosystems. Soil fauna significantly promoted the humification process of forest litter at 3000 m elevation during the deep freezing period; significantly promoted the humification process of forest litter at 3600 m elevation during the pre-freezing period; and significantly inhibited the humification process of meadow litter at 4000 m elevation during the thawing period; while no significant effects were observed at other elevations and periods. The effect of soil fauna on litter humification rate was greater during the early freezing period than during the deep freezing and thawing periods, with the degree of humification reaching its maximum during the deep freezing period. These results indicate that winter warming under climate change scenarios may cause soil fauna to inhibit litter humification, thereby reducing the transformation of litter into soil organic matter.

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Preamble

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Effects of Soil Fauna on Winter Litter Humification Along an Altitudinal Gradient in Cold Ecosystems in Western Sichuan

TAN Yu, YANG Wanqin, LIAO Shu, PENG Yan, LI Jun, WU Fuzhong[1],[2],*

[1]Institute of Ecology and Forestry, Sichuan Agricultural University, Chengdu 611130, China
[2]Key Laboratory of Ecological Forestry Engineering, Chengdu 611130, China

*Corresponding author. E-mail: wufzchina@163.com

Abstract

Foliar litter humification during winter represents a critical stage for soil organic matter formation and carbon sequestration in alpine ecosystems, and may be influenced by soil fauna that maintain activity under harsh freezing conditions. However, this process has received insufficient research attention. This study investigated typical leaf litters from alpine forests and meadows at 3000 m, 3600 m, and 4000 m in the alpine gorge region of western Sichuan using litterbags with different mesh sizes to exclude soil fauna. Following the natural decomposition process, we examined the effects of soil fauna on litter humification during different freeze-thaw periods. The results demonstrated that soil fauna promoted leaf litter humification as temperature decreased, but suppressed it as temperature increased during winter in cold ecosystems. The effects of soil fauna on litter humification were regulated by both altitude and litter species, with the overall influence decreasing with increasing altitude. Soil fauna significantly promoted humification of forest litter at 3000 m during the deep freezing stage and at 3600 m during the onset of freezing, but significantly inhibited humification of meadow litter at 4000 m during the thawing stage. No significant effects were observed at other altitudes and stages. The effects of soil fauna on humification rates were highest during the onset of freezing compared to the deep freezing and thawing stages, with humification peaking during the deep freezing stage. These findings suggest that winter warming under climate change scenarios may lead to soil fauna inhibiting litter humification, thereby affecting the conversion of litter to soil organic matter.

Keywords: humification; soil fauna; leaf litter; tone coefficient; optical density value

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1. Study Area Description

The study area is located in the Miyaluo Nature Reserve, Li County, Aba Tibetan and Qiang Autonomous Prefecture, Sichuan Province, within the transition zone between the eastern Tibetan Plateau and the Sichuan Basin (102.53°-102.57°E, 31.14°-31.19°N). We selected three representative sites at different altitudes:

3000 m (Alpine Forest): Mean annual temperature 3.6°C (ranging from -15.8°C to 24.8°C), mean annual precipitation approximately 850 mm. The seasonal freeze-thaw period occurs from November to March. Dominant tree species include *Abies faxoniana* and *Betula albo-sinensis*, with understory shrubs such as *Fargesia spathacea* and *Berberis julianae*. Soils are brown and dark brown soils developed on limestone and slate, with thin soil layers.

3600 m (Alpine Forest): Mean annual temperature 2.3°C (ranging from -18°C to 23°C), mean annual precipitation approximately 850 mm. Dominant tree species are *Sabina saltuaria* and *Abies faxoniana*, with understory shrubs primarily *Salix paraplesia*. Soils are brown and dark brown soils developed on limestone and slate.

4000 m (Alpine Meadow): Located at the treeline ecotone with mean annual temperature -2 to -4°C (ranging from -8°C to 12.6°C), mean annual precipitation 600–1100 mm. Vegetation consists of alpine meadow species including *Ajania nubigena*, *Carex atrofusca*, *Festuca stapfii*, and *Polygonum viviparum*. Soil type is alpine meadow soil.

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2. Plot Setup and Sample Processing

Based on preliminary surveys, fresh leaf litter was collected from each altitude: *Betula albo-sinensis* and *Abies faxoniana* at 3000 m; *Sabina saltuaria* and *Salix paraplesia* at 3600 m; and *Ajania nubigena* and *Carex atrofusca* at 4000 m. The collected litter was air-dried in the laboratory, and 10 g samples were placed in litterbags of different mesh sizes: 0.04 mm (excluding macro- and meso-fauna) and 2 mm (allowing all soil fauna access). Both surface and bottom layers of the litterbags used 0.04 mm mesh.

Before complete soil freezing in November 2013, we randomly selected homogeneous 5 m × 5 m plots at each site, removed surface litter and debris, and placed litterbags flat on the ground. At each altitude, 36 litterbags were deployed (6 species × 2 mesh sizes × 3 replicates). Additional samples were oven-dried to constant weight at 65°C to determine initial dry mass.

Temperature monitoring was conducted using button temperature loggers placed in specific litterbags and on branches 1.5 m above ground, programmed to record every 2 hours. The seasonal freeze-thaw period was divided into three stages based on local meteorological data: onset of freezing (November

22–December 22, 2013), deep freezing (December 23, 2013–March 9, 2014), and thawing (March 10–April 22, 2014). Parameters including daily average temperature (AT), daytime temperature (ADT), nighttime temperature (ANT), positive accumulated temperature (PAT), negative accumulated temperature (NAT), and number of freeze-thaw cycles (NFTC) were calculated .

Sampling occurred at each critical stage, with three litterbags collected per species per mesh size from each plot. Samples were sealed and returned to the laboratory for air-drying. Dried samples were placed in conical flasks with 100 mL of mixed extractant (0.1 mol/L NaOH + 0.1 mol/L Na P O), shaken for 30 minutes, and filtered. The filtrate was cooled to room temperature for 10 minutes. UV-Vis spectrophotometry (Puxi T1901, Beijing, China) was used to measure absorbance at wavelengths of 400 nm, 465 nm, 600 nm, and 665 nm, from which tone coefficient (ΔlogK) and optical density values (E /E) were calculated.

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3. Data Analysis

The ΔlogK and E /E ratios characterize humus complexity. ΔlogK and E /E are positively correlated with molecular simplicity; larger values indicate simpler molecular structures, while smaller values indicate greater complexity [17,18]. Calculations were as follows:

ΔlogK = log(A /A)
E /E = A /A

where A , A , A , and A represent absorbance at the respective wavelengths.

Statistical analyses were performed using SPSS 20.0 (IBM SPSS Statistics Inc., Chicago, IL, USA) and Origin Pro 8.6 (Origin Lab, Northampton, MA, USA). Paired-samples t-tests compared humification between litterbags with and without soil fauna. Multi-factor ANOVA examined effects of altitude, litter species, and critical stage on ΔlogK and E /E . Significance level was set at P 0.05. Data are presented as means ± standard deviation.

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4. Results and Analysis

4.1 Effects of Soil Fauna on ΔlogK Values

Over the entire winter, soil fauna showed no significant effect on ΔlogK values for six litter types at 3000 m and 3600 m, but promoted humification at 4000 m. However, significant temporal variations existed ($F = 14.17$, $P < 0.001$). At 3600 m, soil fauna significantly promoted ΔlogK values for *Sabina saltuaria* and *Salix paraplesia* during the onset of freezing ($F = 26.33$, $P < 0.001$), while inhibiting values for *Ajania nubigena* and *Carex atrofusca* during the thawing stage. No significant effects were observed for other species or stages

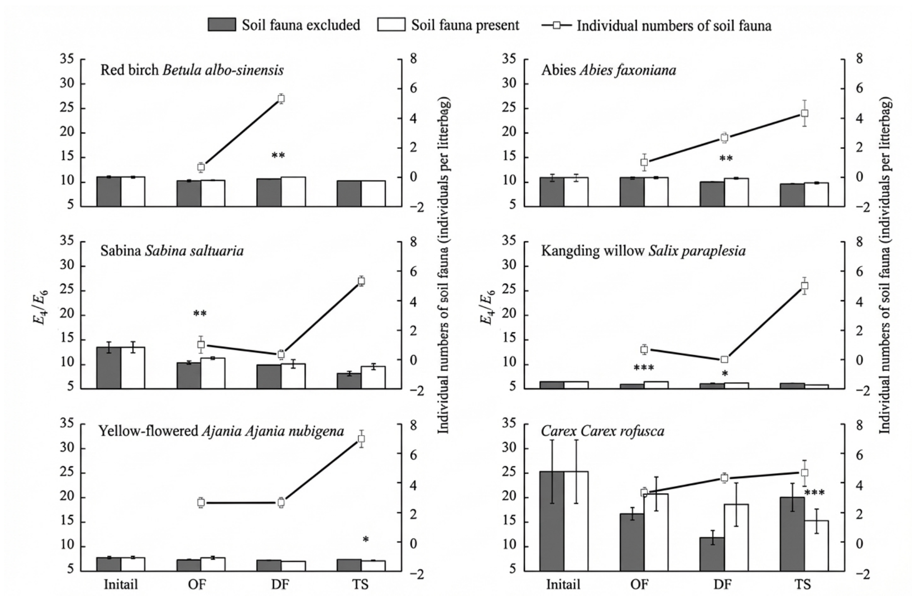

Figure 1: Figure 2

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4.2 Effects of Soil Fauna on E /E Values

Soil fauna significantly affected E /E values across altitudes and species. They promoted E /E for four litter types at 3000 m and 3600 m during freezing onset, but inhibited values for *Ajania nubigena* and *Carex atrofusca* at 4000 m during thawing. During deep freezing, soil fauna significantly promoted E /E for *Betula albo-sinensis*, *Abies faxoniana*, and *Salix paraplesia* at 3000 m, but inhibited values for *Ajania nubigena* and *Carex atrofusca* at 4000 m. Effects were most pronounced during freezing onset compared to deep freezing and thawing stages [FIGURE:3].

4.3 Multi-Factor Analysis

Three-way ANOVA revealed that soil fauna, litter species, and critical stage significantly influenced ΔlogK and E /E values, with significant interactions between factors . The effect of soil fauna on humification decreased significantly with increasing altitude ($F = 18.48$, $P < 0.001$). Soil fauna significantly affected ΔlogK at 3000 m ($F = 4.66$, $P < 0.05$) and E /E at both 3000 m ($F = 15.25$, $P < 0.001$) and 4000 m ($F = 4.94$, $P < 0.05$). Litter species showed highly significant effects on both parameters across all altitudes.

Correlation analysis indicated that during the onset of freezing, the number of freeze-thaw cycles was significantly positively correlated with ΔlogK values at 3000 m ($P < 0.05$).

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5. Discussion and Conclusion

Soil fauna, defined as animals that spend part of their life cycle in soil and influence soil properties [19], feed on soil organic matter and other organisms, positively contributing to litter degradation and humus formation [20,21]. This study reveals that soil fauna effects on litter humification vary across critical winter stages and altitudes, promoting humification as temperature decreases while suppressing it as temperature increases.

The seasonal freeze-thaw cycle is intense in alpine forests [9,10]. Humification was faster during the onset of freezing compared to deep freezing and thawing stages, with ΔlogK reaching its lowest value (indicating maximum humification) during deep freezing. During freezing onset—the peak litterfall period—fresh litter provides abundant food resources [22] while forming snow cover insulates and protects microbial activity [6]. This creates favorable conditions for soil fauna and microorganisms to drive humification [23,24]. The significant promotion of humification for *Sabina saltuaria* and *Salix paraplesia* at 3600 m during this stage likely reflects these synergistic effects.

During deep freezing, snow cover provides thermal insulation, maintaining conditions suitable for soil fauna and microbial activity [10], thereby promoting humification. At 3000 m, litterbag temperatures were higher than at 3600 m and 4000 m, creating more favorable conditions for soil fauna. The differential effects on coniferous versus broadleaf litter reflect how fauna fragmentation influences decomposition [25]. Vegetation differences along the altitudinal gradient create distinct soil fauna and microbial communities [26], resulting in species-specific humification responses.

During thawing, increased temperature and intense freeze-thaw cycles degrade easily decomposable compounds [27]. Soil fauna may ingest humic substances, and enzymes in their guts can degrade humus, inhibiting humification. This effect was particularly significant for meadow species (*Ajania nubigena* and *Carex atrofusca*) at 4000 m, while forest litter at lower altitudes showed less response due to lower freeze-thaw intensity and greater canopy cover.

Overall, soil fauna promoted humification at 3000 m and 3600 m but inhibited it at 4000 m, with significant species-specific differences. These results demonstrate that soil fauna and microorganisms exhibit special adaptations to soil environmental changes, influencing mineralization and humification processes [28]. Winter temperature changes and freeze-thaw cycles indirectly affect humus accumulation. As temperatures decrease, soil fauna activity may promote humification and carbon sequestration; as temperatures increase, fauna may inhibit humification and enhance carbon release. These findings provide new insights into soil organic matter formation and carbon budgeting in cold ecosystems under climate change scenarios.

References

[1] Sollins P, Homann P, Caldwell B A. Stabilization and destabilization of soil organic matter: mechanisms and controls. *Geoderma*, 1996, 74(1/2): 65-105.

[2] Ono K, Hiradate S, Morita S, Ohse K, Hirai K. Humification processes of needle litters on forest floors in Japanese cedar (*Cryptomeria japonica*) and Hinoki cypress (*Chamaecyparis obtusa*) plantations in Japan. *Plant and Soil*, 2011, 338(1/2): 171-181.

[3] Forest Soil Ecology. Chengdu: Sichuan Science and Technology Press, 2006.

[4] Swift M J, Heal O W, Anderson J M. *Decomposition in Terrestrial Ecosystems*. Oxford: Blackwell Scientific, 1979.

[5] Wolters V. Invertebrate control of soil organic matter stability. *Biology and Fertility of Soils*, 2000, 31(1): 1-19.

[6] Wu F Z, Yang W Q, Zhang J, Deng R J. Litter decomposition in two subalpine forests during the freeze-thaw season. *Acta Oecologica*, 2010, 36(1): 135-140.

[7] Effects of soil organisms on soil organic carbon stability. *Advances in Earth Science*, 2007, 22(2): 152-158.

[8] Ekschmitt K, Liu M Q, Vetter S, Fox O, Wolters V. Strategies used by soil biota to overcome soil organic matter stability—why is dead organic matter left over in the soil? *Geoderma*, 2005, 128(1/2): 167-176.

[9] Hydrology of Cold Regions in China. Beijing: Science Press, 2000.

[10] Campbell J L, Mitchell M J, Groffman P M, Christenson L M, Hardy J P. Winter in northeastern North America: a critical period for ecological processes. *Frontiers in Ecology and the Environment*, 2005, 3(6): 314-322.

[11] Diversity of macro-soil fauna communities and their response to seasonal freeze-thaw in subalpine/alpine forests of western Sichuan. *Biodiversity Science*, 2012, 20(2): 215-223.

[12] Plant growth and ecophysiological characteristics along altitudinal gradients. *Chinese Journal of Plant Ecology*, 2009, 18(2): 722-730.

[13] Yang W Q, Wang K Y, Kellomäki S, Gong H D. Litter dynamics of three subalpine forests in western Sichuan. *Pedosphere*, 2005, 15(5): 653-659.

[14] Effects of snow cover removal on soil temperature and carbon-phosphorus dynamics in an alpine forest of western Sichuan. *Chinese Journal of Applied Ecology*, 2011, 22(10): 2553-2559.

[15] Wardle D A, Bardgett R D, Klironomos J N, Setälä H, van der Putten W H, Wall D H. Ecological linkages between aboveground and belowground biota. *Science*, 2004, 304(5677): 1629-1633.

[16] Ni X Y, Yang W Q, Li H, Xu L Y, He J, Tan B, Wu F Z. The responses of early foliar litter humification to reduced snow cover during winter in an alpine forest. *Canadian Journal of Soil Science*, 2014, 94(4): 453-461.

[17] Richard G, Guyot G, Aguer J P, Ter Halle A, Trubetskaya O E, Trubetskoi O A. Role of fractionation in studying the photochemical properties of humic substances. *Russian Journal of General Chemistry*, 2008, 78(11): 2265-2272.

[18] Study on soil humus fractionation. *Acta Pedologica Sinica*, 2004, 35(6): 706-709.

[19] Soil Fauna of China. Beijing: Science Press, 2000: 1-2.

[20] Application of 14C tracer methods in studying soil organic carbon transformation by geophagous macrofauna. *Soils*, 2008, 40(6): 863-871.

[21] Effects of microbial inoculants on humus composition during *Dicranopteris* straw decomposition. *Chinese Journal of Tropical Crops*, 2015, 36(4): 719-723.

[22] Effects of snow patches on humic and fulvic acid accumulation during litter humification in alpine forests. *Scientia Silvae Sinicae*, 2014, 51(5): 1138-1152.

[23] Thermodynamic stability of soil humic and fulvic acids and their driving factors. *Soils*, 2010, 47(1): 71-76.

[24] Cotrufo M F, Wallenstein M D, Boot C M, Denef K, Paul E. The microbial efficiency-matrix stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology*, 2013, 19(4): 988-995.

[25] Briones M J I, Ineson P, Piearce T G. Effects of climate change on soil fauna; responses of enchytraeids, diptera larvae and tardigrades in a transplant experiment. *Applied Soil Ecology*, 1997, 6(2): 117-134.

[26] Characteristics of soil fauna communities in typical vegetation types on the eastern slope of Mt. Gongga. *Acta Ecologica Sinica*, 2015, 35(7): 2295-2307.

[27] Effects of snow patches on winter humification of three litter types in a subalpine forest of western Sichuan. *Chinese Journal of Plant Ecology*, 2014, 38(6): 540-549.

[28] Changes in soil organic carbon and humus carbon and their microbial effects in alpine steppe soils. *Scientia Silvae Sinicae*, 2014, 51(4): 834-844.

Figures

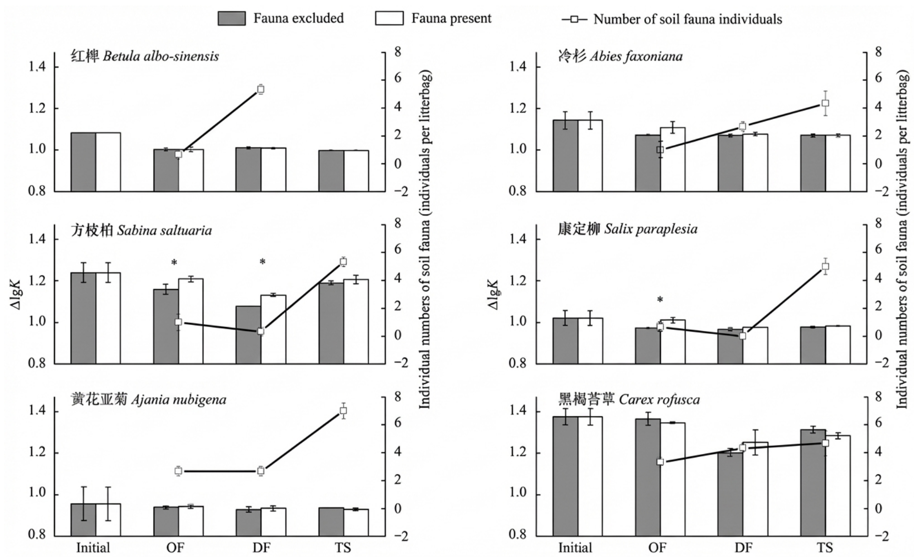

Figure 2: Figure 1

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