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Water, Rather than Temperature, Dominantly Impacts How Soil Fauna Affect Dissolved Carbon and Nitrogen Release from Fresh Litter during Early Litter Decomposition

Shu Liao [1,†], Xiangyin Ni [1,2,3,†], Wanqin Yang [1,4], Han Li [1], Bin Wang [5], Changkun Fu [1], Zhenfeng Xu [1,4], Bo Tan [1,4] and Fuzhong Wu [1,4,*]

[1] Long-Term Research Station of Alpine Forest Ecosystems, Key Laboratory of Ecological Forestry Engineering, Institute of Ecology and Forestry, Sichuan Agricultural University, Chengdu 611130, China; liaoshu_224@163.com (S.L.); nixiangyin_922@163.com (X.N.); scyangwq@163.com (W.Y.); hannahlisc@163.com (H.L.); fckgood@sina.com (C.F.); xuzf@sicau.edu.cn (Z.X.); bobotan1984@163.com (B.T.)

[2] Advanced Science Research Center, Brooklyn College of the City University of New York, New York, NY 10031, USA

[3] Department of Earth and Environmental Sciences, Brooklyn College of The City University of New York, New York, NY 11210, USA

[4] Collaborative Innovation Center of Ecological Security in the Upper Reaches of the Yangze River, Chengdu 611130, China

[5] Laboratory of Forestry, Department of Forest and Water Management, Ghent University, Geraardsbergsesteenweg 267, BE-9090 Gontrode (Melle), Belgium; bin.wang@ugent.be

* Correspondence: wufzchina@163.com; Tel.: +86-28-86290957

† These authors contributed equally to this work.

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Abstract: Longstanding observations suggest that dissolved materials are lost from fresh litter through leaching, but the role of soil fauna in controlling this process has been poorly documented. In this study, a litterbag experiment employing litterbags with different mesh sizes (3 mm to permit soil fauna access and 0.04 mm to exclude fauna access) was conducted in three habitats (arid valley, ecotone and subalpine forest) with changes in climate and vegetation types to evaluate the effects of soil fauna on the concentrations of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) during the first year of decomposition. The results showed that the individual density and community abundance of soil fauna greatly varied among these habitats, but Prostigmata, Isotomidae and Oribatida were the dominant soil invertebrates. At the end of the experiment, the mass remaining of foliar litter ranged from 58% for shrub litter to 77% for birch litter, and the DOC and TDN concentrations decreased to 54%–85% and increased to 34%–269%, respectively, when soil fauna were not present. The effects of soil fauna on the concentrations of both DOC and TDN in foliar litter were greater in the subalpine forest (wetter but colder) during the winter and in the arid valley (warmer but drier) during the growing season, and this effect was positively correlated with water content. Moreover, the effects of fauna on DOC and TDN concentrations were greater for high-quality litter and were related to the C/N ratio. These results suggest that water, rather than temperature, dominates how fauna affect the release of dissolved substances from fresh litter.

Keywords: soil fauna; dissolved organic carbon; dissolved nitrogen; subalpine forest

1. Introduction

In the last decades, numerous observations have greatly advanced our understanding of how large amounts of labile substances are released from plant litter during the early stage of decomposition [1,2]. Empirical evidence suggests that these soluble compounds, such as dissolved organic carbon (DOC) and total dissolved nitrogen (TDN), are primarily released from freshly shed litter through hydrological leaching accompanied by rainfall or snow melt [3] and then move into the soil horizon or aquatic ecosystems [4]. However, Fröberg et al. found that large amounts of litter-derived DOC were retained and consumed on the soil surface without being transferred into the mineral soils [5]. Versini et al. also demonstrated that the low DOC and TDN concentrations derived from fresh litter can percolate deep into mineral soil [6]. These findings imply that dissolved carbon and nitrogen are important sources of carbon and nutrients for soil decomposers and may be quickly assimilated by soil organisms living on the litter surface [4,7] because of the low molecular weight of the litter leachates [8]. However, information regarding how soil fauna contribute to the release of dissolved carbon and nitrogen from plant litter is limited.

Faunal exclusion experiments using litterbags with different mesh sizes to remove fauna with specified size classes [9] have suggested that soil invertebrates greatly contribute to litter decay [10] by directly fragmenting and consuming the litter materials or by indirectly modifying bacterial/fungal colonization [11]. García-Palacios et al. synthesized global data and quantitatively found that soil fauna consistently promote litter decomposition by an average of 37% at the global scale, but the faunal effect varies between biomes, with a stronger positive effect of soil fauna in warmer and wetter ecosystems and a weaker effect in cold and arid ecosystems [12]. Field experiments also demonstrated that the faunal effect on litter decomposition is greater at wetter sites [13]. Although this different influence of soil fauna may be more sensitive for dissolved substances because of their soluble properties, current information is not available.

Litter quality is related to the palatability of soil fauna and the degree of fragmentation of litter materials [14,15]. High-quality litter contains more easily degradable compounds and is expected to decompose faster [16,17]. However, some researchers did not find significant differences in the decomposition rate between relatively high- and low-quality litter that were affected by soil fauna [13]. Isotopic labeling studies have suggested that most soil fauna settle upon the litter surface [18] and preferentially feed on available nutrients rather than the recalcitrant plant detritus itself [7,19]; thus, compared with the total litter materials, the dissolved parts (i.e., DOC and TDN) in newly shed litter may be affected by soil fauna, and such an effect may be greater for high-quality litter.

To address the effects of soil fauna on the shifts in DOC and TDN in fresh litter, we conducted a field experiment using litterbags with different mesh sizes. Faunal abundance varies between seasons with changes in temperature and rainfall [20,21]; moreover, hydrological leaching that contributes to DOC and TDN release normally occurs during the growing season [22,23]. Whether the faunal effect interacts with this process is unclear. Meanwhile, greater temperatures and higher water contents both could promote the effects of soil fauna [12,13], but it is unclear which factor dominates the effects of fauna on DOC and TDN. In addition, high-quality litter with rich labile substances may be affected more by soil fauna because most decomposers are able to consume it [24], but the current information is far from clear. To evaluate the variations of the effects of fauna on DOC and TDN between seasons, habitats and substrate quality levels, we selected three experimental sites (arid valley, ecotone and subalpine forest) with contrasting temperatures and moisture regimes along an altitudinal gradient. With increasing elevation, the climate becomes colder and wetter and the vegetation becomes more abundant. In each habitat, we studied two litter species (one high-quality litter with a low C/N ratio and another low-quality litter with a higher C/N ratio) to compare the different faunal effects between litter qualities. To avoid the home-field advantage [24,25] that would result from the diverse climate and vegetation types between the habitats, only the original trees or shrubs were considered in this study. Specifically, we hypothesized the following: (a) The effects of soil fauna on DOC and TDN during the growing season are greater than during the winter because of the richer decomposer community and higher litter leachates; (b) The faunal effect is greater in the subalpine forest than in the arid valley due to the wetter environmental conditions even though the temperature is lower; (c) The faunal effect on DOC and TDN is greater for high-quality litter.

2. Materials and Methods

2.1. Site Description

This study was conducted in an ecotone and its adjacent arid valley and subalpine forest along the Minjiang River, which is located on the eastern Tibetan Plateau and is a major branch of the upper reaches of the Yangtze River.

The arid valley site (31°34′ N, 103°21′ E, 1563 m a.s.l.) is near the river. The mean annual temperature is 11.2 °C, and the highest and lowest monthly temperatures 20.8 °C and −0.4 °C occur in July and January, respectively. The mean annual precipitation is only 374 mm, but evaporation is very high (1332 mm), thus most of the original vegetation is resistant to water deficit conditions and exists as shrubs, such as *Campylotropis macrocarpa* (Bge.) Rehd. and *Sophora davidii* (Franch.) Skeels. Some drought-resistant trees were artificially planted to conserve the water and soil, but these seedlings grow very slowly. For instance, most of the cypress (*Cupressus chengiana* S. Y. Hu) trees at the site are only 1.6 m tall after 9 years of forestation, although they represent a higher fraction of the total biomass compared with other vegetation in this area. The thin soils are classified as Aridisols (WRB taxonomy) [26] and are mixed with gravel; thus, it is difficult to distinguish between the soil organic layer and mineral layer.

The ecotone site (31°33′ N, 103°26′ E, 2158 m a.s.l.) is located in an oak-cypress mixed secondary forest that represents a transitional area from the arid valley to the subalpine forest along the river basin. The mean annual temperature and precipitation are 11.3 °C and 803 mm, respectively. The dominant trees are cypress and oak (*Quercus baronii* var. baronii), and the main understory shrubs are *Sophora davidii* (Franch.) Skeels and *Berberis wilsonae* var. wilsonae Although the temperature and plant diversity are greater than those in the arid valley, the low rainfall and high evaporation also limit plant growth, and the DBH (diameter at breast height) of most of the trees is less than 20 cm. The soils in this forest are categorized as Alfisols, and the soil organic layer is thin.

The subalpine forest site (31°33′ N, 102°56′ E, 3028 m a.s.l.) is located in a birch-fir mixed forest, with a mean annual temperature of 3.6 °C, and maximum and minimum monthly temperatures of 24.8 °C and −15.8 °C in July and January, respectively. The mean annual precipitation is approximately 850 mm, which includes the seasonal snow fall [27]. The dominant trees are fir (*Abies faxoniana* Rehd.) and birch (*Betula albosinensis* Burk.), and the main shrubs are *Fargesia spathacea* Franch. and *Hippophae rhamnoides* L. The soils are categorized as Cambisols, and some detailed information is given in Table 1.

Table 1. Geology and soil properties of the soil organic layers at the experimental sites.

Site	Aspect	Slope (°)	Soil Bulk Density	pH	C (mg g^{-1})	N (mg g^{-1})	P (mg g^{-1})	Duration of Soil Freeze-Thaw
Arid valley	NW 327°	36	1.76	7.50	25.20	0.84	0.65	10 December to 10 February
Ecotone	NE 53°	23	1.49	5.70	47.20	2.91	0.38	21 November to 13 March
Subalpine forest	SE 128°	3	0.62	5.90	161.4	8.10	0.90	25 October to 24 April

C: carbon, N: nitrogen, P: phosphorus.

2.2. Experimental Design

Litterbags with different mesh sizes are commonly used for studying the effects of soil fauna on litter decomposition [28]. Researchers have shown that a 5 mm mesh size allows all soil fauna (micro-, meso- and macro-fauna) to reach the litter in the bag [11]. However, keeping the samples from escaping the litterbags is difficult because some needles are smaller than 5 mm; thus, accurately estimating mass loss is challenging. Our previous research found that few soil fauna in this region are larger than 3 mm and that macro-fauna (such as earthworms, which are normally present in temperate forests and grassland) were not observed. In contrast, some meso-fauna, such as oribatid mites and Collembola, were dominant in the soil animal community [21]. Therefore, we used 3 mm mesh litterbags to allow outside soil fauna to access the litterbags and 0.04 mm mesh litterbags to exclude all macro-, meso- and micro-fauna.

At each site, three 5 m × 5 m blocks separated by at least 100 m and with homogeneous aspect, slope and tree type were treated as replicates. Each block was divided into four plots: two of the plots were considered soil faunal plots (SF) and the other two plots were considered control plots (no soil fauna, NSF); both the faunal and control plots contained two litter species. A total of 36 plots (3 habitats × 3 replicate blocks × 2 treatments × 2 species) were established (Figure 1).

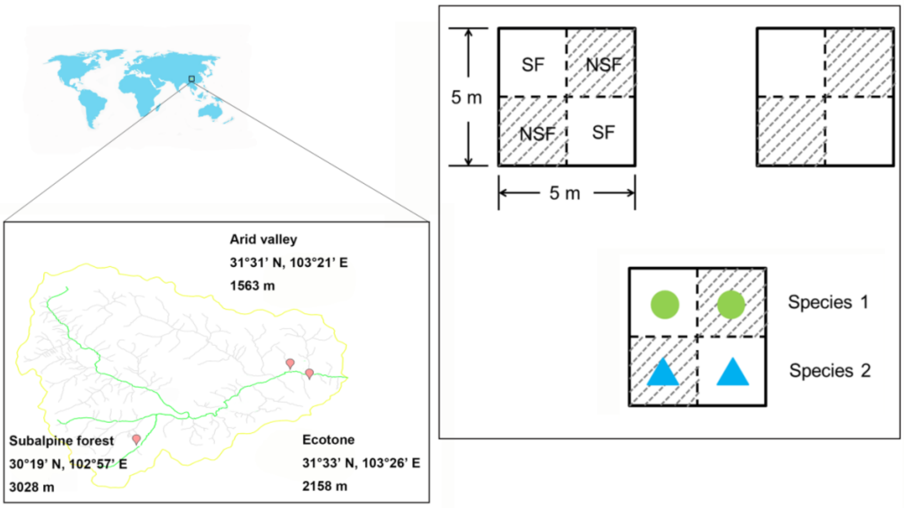

Figure 1. Experimental design of the study. SF: soil fauna; NSF: no soil fauna.

In October 2013, freshly senesced foliar litter from the dominant species and with different initial qualities (i.e., C/N ratio; Table S1) in each habitat were carefully collected from the soil surfaces of the forest and valley. Specifically, foliar litter from planted cypress (ca. 9 years old) and *C. macrocarpa* shrubs was collected in the arid valley, foliar litter from native cypress and oak was collected at the ecotone site, and foliar litter from fir and birch was harvested from the subalpine forest. All samples were brought back to the laboratory and air dried at room temperature for two weeks. Samples equivalent to 10 g of their oven-dried counterparts were placed in two types of nylon litterbags (20 cm × 20 cm in size): a fine mesh litterbag (0.04 mm on both sides) and a coarse mesh litterbag (3 mm on the top and 0.04 mm on the bottom). A total of 216 litterbags (36 plots × 2 sampling dates × 3 litterbags per time per plot) were transferred to the corresponding plots and their corners were fixed to the soil surface by nylon lines to prevent the litterbags from being blown away by winds on 12 November 2013. The temperatures on the litter surfaces in each habitat were recorded every 2 h using data loggers (iButton DS1923-F5, Maxim/Dallas Semiconductor, Sunnyvale, CA, USA) (Figure 2; Table S2).

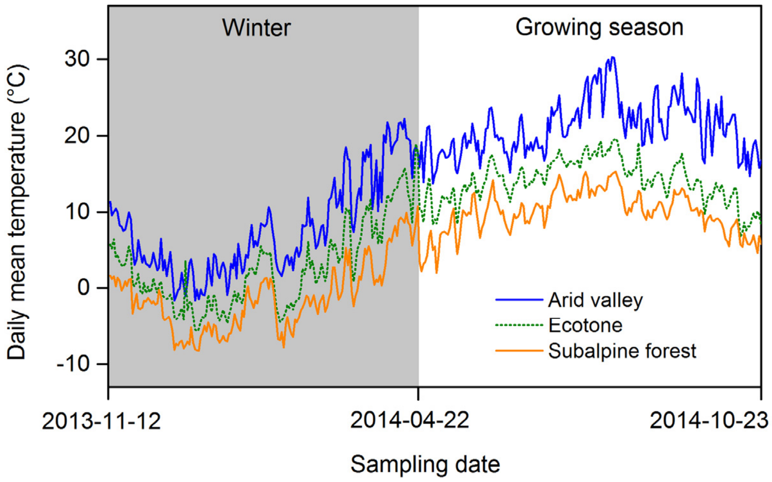

Figure 2. Daily mean temperature at the litter surface in the experimental sites. This study was divided into the winter (162 days) and growing seasons (184 days) according to our previous long-term observations.

2.3. Sample Collection and Analysis

Winter in the studied regions is a long period [29], and heavy snowfall and earthquakes during the winter make sampling challenging. Thus, we collected samples at the end of winter and at the end of the growing season in these regions according to our previous observations [30–33]. Three litterbags were randomly harvested from each plot on 22 April and 23 October 2014, 162 and 346 days after the initial setup, respectively. Unfortunately, the winds in the arid valley are strong, and the litterbags in the control plot that was projected to be sampled on 22 April 2014, were lost.

One litterbag was carefully picked up, immediately placed into a light-shaded sealed bag and then taken to the laboratory to identify the individual soil fauna as described by Tan et al. [21]. Macro-fauna were picked up by hand, and meso-/micro-fauna and damp living fauna were separated from the litter sample by Tullgren and Baermann, respectively. All collected soil fauna were placed in 75% alcohol and then classified using a microscope and identified to the family level as described by Yin et al. [34] (Table S3). One sample was oven-dried at 105 °C to constant weight [35] to measure the water content (Figure S1) and was subjected to combustion at 550 °C for four hours to measure the ash-free mass [36]. Another sample was air dried and used to detect the concentrations of DOC and TDN [37]. Specifically, 0.5 g of the air dried subsample was transferred to a screw-cap tube that was then filled with 40 mL of deionized water and extracted for one hour at 25 °C [4]. The leachate was passed through 0.45 µm filters [8] and then used to determine the concentrations of DOC and TDN using a TOC analyzer (multi N/C 2100, Analytik Jena, Thüringen, Germany) and the Kjeldahl method (KDN, Top Ltd., Zhejiang, China), respectively. The concentrations of DOC and TDN were calculated based on dry mass. All data used in this study were deposited in supplementary datasets (Data S1–S6). Notably, the soluble fractions are challenging to study because of the complexities of tracing both the formation of new solutes and the disappearance of the same solutes because of leaching and metabolism [1]; thus, we only discuss the concentration dynamics of DOC and TDN in this study.

2.4. Data Calculations and Statistical Analyses

Soil freezing and thawing are important winter ecological processes that impact ecosystem functions in these regions [38]. One freeze-thaw cycle is considered complete when the threshold of 0 °C is crossed twice for 3 or more hours [39]. We calculated the frequency of freeze-thaw cycle based on the total number of freeze-thaw cycles and the day of a certain period (Table S2).

All individual fauna in the soil fauna treatment were categorized in different groups. The Shannon-Wiener, Simpson, Pielou and Margalef indexes were then calculated to represent the diversity of the faunal communities.

Two-way analysis of variance (ANOVA) was used to test the effects of litter species, soil fauna and their interactions on the concentrations of DOC and TDN on each sampling date. One-way ANOVA with multiple comparisons using Tukey's honestly significant difference (HSD) post-hoc test was employed to test for significant differences among the litter species at the $p = 0.05$ level. Homogeneity of variance was determined before conducting the ANOVAs, and the data were logarithmically transformed when required. A pairwise *t*-test was conducted to determine whether the variables (mass remaining, water content, and DOC and TDN concentrations) were significantly different between the soil fauna and no-soil fauna treatments at three *p*-levels (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). These analyses were performed in MATLAB R2012a (MathWorks Inc., Natick, MA, USA).

3. Results

3.1. Soil Fauna

Totals of 37, 140 and 222 individuals of soil fauna were observed in the arid valley, ecotone and subalpine forest, respectively, during the entire study period (Table 2). Prostigmata was the dominant fauna group in the arid valley, and Prostigmata, Isotomidae and Oribatida were the dominant soil animals in both the ecotone and subalpine forest (Table S3). Individual number of soil fauna significantly ($p < 0.05$) varied between seasons ($f = 44.9$, $p < 0.001$; Table S4) and litter species ($f = 5.3$, $p = 0.002$). The numbers of individual fauna observed on October 23 were significantly ($p < 0.05$) higher than those observed on April 22, except for the oak litter. The number of individual fauna in the birch litter was significantly ($p < 0.05$) greater than that in the fir litter, but no significant ($p > 0.05$) differences were found between the two types of litter from the same site.

The Shannon-Wiener, Simpson, Pielou and Margalef indexes of soil fauna also varied between seasons (all $p < 0.05$; Table S4). Compared with the winter, significantly ($p < 0.05$) higher Shannon-Wiener, Simpson and Margalef indexes and a lower Pielou index of soil fauna were observed for the birch litter during the growing season (Table S5). The Simpson index of soil fauna was significantly ($p < 0.05$) greater for the birch litter than for the fir litter during the growing season.

Table 2. Number of individuals (±SE, $n = 3$) of soil fauna observed in the 3 mm mesh litterbag during the winter and growing seasons.

Period	Arid Valley		Ecotone		Subalpine Forest	
	Planted Cypress	Shrub	Native Cypress	Oak	Fir	Birch
Winter	N/A	0 ± 0 bB	12.0 ± 2.5 bAB	3.0 ± 1.7 aB	4.7 ± 2.2 bB	19.3 ± 3.8 bA
Growing season	26.7 ± 12.7 AB	10.7 ± 4.7 aB	72.7 ± 5.8 aAB	52.0 ± 26.9 aAB	83.0 ± 16.5 aAB	114.7 ± 32.4 aA

Different lowercase letters in the same column indicate significant ($p < 0.05$) differences between seasons for a certain litter species, and capital letters in the same row indicate significant ($p < 0.05$) differences among litter species for a certain season. N/A indicates data are not available because some samples were lost due to strong winds.

3.2. Mass Remaining

After one year of decomposition, the lowest mass remaining was observed for shrub litter (58%), followed by native cypress (62%), oak (67%), planted cypress (72%), fir (76%), and birch (77%) for the control. During the winter, significantly ($p < 0.05$) lower remaining masses were observed in the soil fauna treatments for shrub, native cypress, oak and fir litter (Figure 3). However, compared with the control, lower remaining masses were observed in the soil fauna treatments for planted and native cypress litter (both $p < 0.01$) during the growing season.

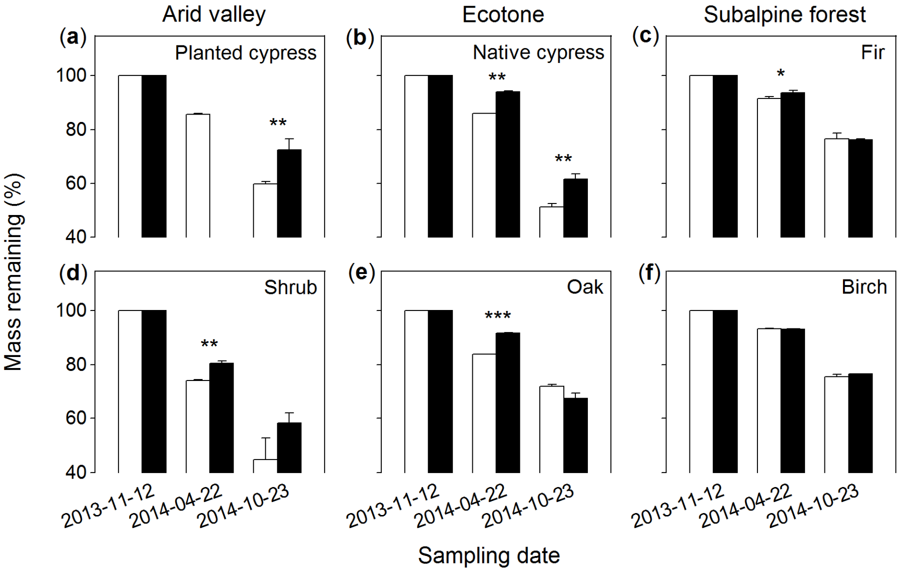

Figure 3. Remaining mass (±SE, $n = 3$) in the foliar litter with (white) and without (black) soil fauna. (**a**) planted cypress; (**b**) native cypress; (**c**) fir; (**d**) shrub; (**e**) oak; (**f**) birch. The designated sample of planted cypress litter on 22 April 2014, was lost due to strong winds; thus, these data are missing. Values with *, ** and *** are significantly different between the treatments at $p = 0.05$, 0.01 and 0.001 levels, respectively.

3.3. Dissolved Organic Carbon

Considerable variations in DOC concentrations were observed among the litter species during both the winter ($f = 456.6$, $p < 0.001$) and growing season ($f = 40.7$, $p < 0.001$; Table 3). All litter species showed decreasing trends in DOC concentrations after one year of decomposition that ranged from 54% for birch litter (Figure 4f) to 85% for fir litter (Figure 4c) in the treatments without fauna (Table 4). However, the native cypress litter exhibited a greater DOC concentration during the winter (Figure 3b). In addition, the two-way ANOVA results indicated that the effect of soil fauna on DOC concentration was not significant ($f = 0.04$, $p = 0.84$) during the winter but was significant ($f = 42.9$, $p < 0.001$) during the growing season. Lower DOC concentrations were observed in the soil fauna treatments with native cypress (15%) and fir litter (33%) during the winter and for in the soil fauna treatments with shrub (69%) and oak litter (35%) during the growing season.

The DOC concentration was negatively correlated with the accumulated mass loss through a quadratic relationship ($R^2 = 0.42$, $f = 89.6$, $p < 0.001$; Figure 5a) but positively correlated with the carbon content ($R^2 = 0.036$, $p = 0.044$; Figure S2a) and C/N ratio ($R^2 = 0.43$, $p < 0.001$; Figure S2b) of the foliar litter. Moreover, the DOC concentration was negatively related to the individual number ($R^2 = 0.34$, $p = 0.041$; Figure S3a) and Simpson index ($R^2 = 0.53$, $p < 0.001$; Figure S3c) of soil fauna.

Table 3. Levels of significance from the two-way ANOVA for comparing the effects of litter species, soil fauna and their interactions on the concentrations of dissolved organic carbon and total dissolved nitrogen in the foliar litter during the winter and growing seasons.

Period	**Concentration of Dissolved Organic Carbon**									**Concentration of Total Dissolved Nitrogen**								
	Litter Species			**Soil Fauna**			**Litter Species × Soil Fauna**			**Litter Species**			**Soil Fauna**			**Litter Species × Soil Fauna**		
	df	***f* value**	***p* value**	***df***	***f* value**	***p* value**	***df***	***f* value**	***p* value**	***df***	***f* value**	***p* value**	***df***	***f* value**	***p* value**	***df***	***f* value**	***p* value**
Winter	5	456.6	<0.001	1	0.04	0.84	5	66.7	<0.001	5	166.8	<0.001	1	0.5	0.49	5	62.4	<0.001
Growing season	5	40.7	<0.001	1	42.9	<0.001	5	26.6	<0.001	5	83.2	<0.001	1	7.2	0.013	5	24.3	<0.001

$n = 36$ for the data from each sampling date.

Table 4. Percent change (%, SEs in parentheses) of the mass remaining and dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) concentrations after one year of decomposition compared with the initial values.

With/Without Soil Fauna	**Arid Valley**		**Ecotone**		**Subalpine Forest**	
	Planted Cypress	**Shrub**	**Native Cypress**	**Oak**	**Fir**	**Birch**
			Mass remaining (%)			
Soil fauna	−40 (1.0) aAB	−55 (8.1) aA	−49 (1.3) bA	−28 (0.8) aB	−23 (2.1) aB	−24 (0.8) aB
No soil fauna	−28 (4.2) aB	−42 (3.7) aA	−38 (2.0) aAB	−33 (1.9) aAB	−24 (0.4) aB	−23 (0.06) aB
			DOC concentration (mg g^{-1})			
Soil fauna	−64 (0.1) aC	−93 (0.5) bA	−64 (3.1) aC	−85 (0.5) bB	−85 (0.2) aB	−40 (1.4) aD
No soil fauna	−57 (2.5) aC	−79 (1.4) aAB	−70 (2.4) bB	−77 (0.6) aAB	−85 (0.09) aA	−54 (3.3) aC
			TDN concentration (μg g^{-1})			
Soil fauna	+92 (4.1) aC	−46 (4.7) bD	+241 (13) aA	+169 (2.0) bB	+98 (11.7) aC	+95 (5.7) aC
No soil fauna	+67 (9.9) aC	+34 (9.0) aC	+151 (6.5) bB	+269 (3.1) aA	+58 (7.6) bC	+77 (14) aC

Different lowercase letters in the same column indicate significant ($p < 0.05$) differences between the treatments for a certain litter species, and different capital letters in the same row indicate significant ($p < 0.05$) differences among the litter species for a certain treatment.

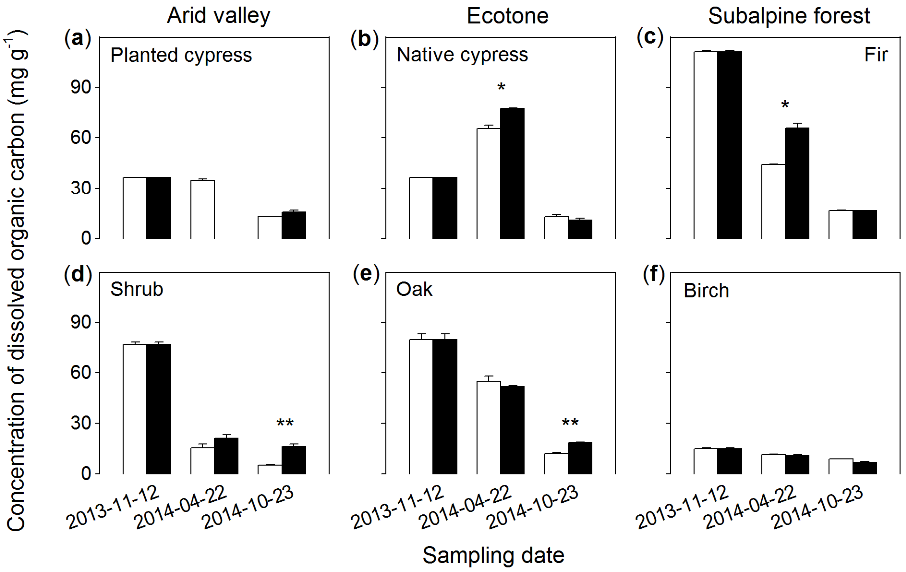

Figure 4. Concentrations (±SE, $n = 3$) of dissolved organic carbon in the foliar litter with (white) and without (black) soil fauna. (**a**) planted cypress; (**b**) native cypress; (**c**) fir; (**d**) shrub; (**e**) oak; (**f**) birch. The designated samples of planted cypress litter on 22 April 2014, were lost due to strong winds; thus, this data is missing. Values with * and ** are significantly different between the treatments at the $p = 0.05$ and 0.01 levels, respectively.

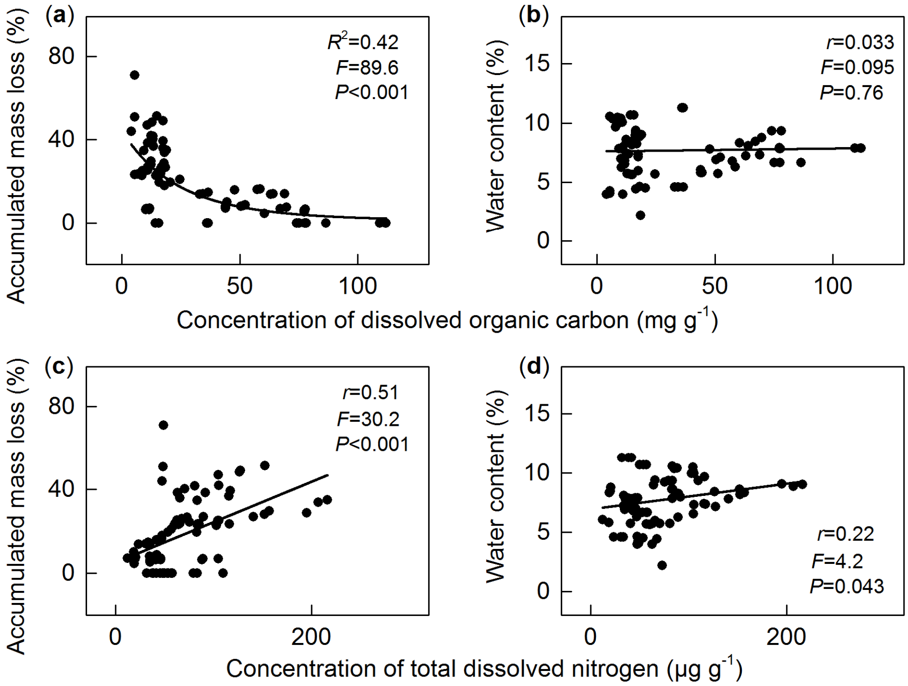

Figure 5. Relationships between the concentrations of (**a**,**b**) dissolved organic carbon and (**c**,**d**) total dissolved nitrogen and the accumulated mass loss and water content in the different types of foliar litter.

3.4. Total Dissolved Nitrogen

The TDN concentrations varied widely among the litter species during the winter and growing season (both $p < 0.001$; Table 3). However, the TDN concentrations decreased during the winter and substantially increased during the growing season for all the litter species except for the birch litter, which consistently increased during the entire year (Figure 6f), resulting in greater TDN concentrations of 34% for shrub litter to 269% for oak litter in the control at the end of the experiment (Table 4). The two-way ANOVA results indicated that the effects of soil fauna on the TDN concentration were not significant ($f = 0.5$, $p = 0.49$; Table 3) during the winter but were significant ($f = 7.2$, $p = 0.013$) during the growing season. Compared with the control, significantly ($p < 0.01$) lower TDN concentrations were observed for birch litter (53%) during the winter and for shrub (60%) and oak litter (27%) during the growing season (Figure 6). However, the TDN concentrations in the native cypress litter during the growing season were significant higher (37%, $p < 0.05$) in the soil fauna treatment than in the control (Figure 6b).

The TDN concentration was positively correlated with the accumulated mass loss ($r = 0.51$, $f = 30.2$, $p < 0.001$; Figure 5c), water content ($r = 0.22$, $f = 4.2$, $p = 0.043$; Figure 5d) and nitrogen content ($r = 0.15$, $p < 0.001$; Figure S2c) in the foliar litter. However, the TDN concentration was negatively correlated with the C/N ratio of the foliar litter through a quadratic relationship ($R^2 = 0.57$, $p < 0.001$; Figure S2d). Linear regression analyses also indicated that the TDN concentration was positively correlated with the individual number ($p < 0.001$), Shannon-Wiener ($p < 0.001$), Simpson ($p < 0.001$) and Margalef indexes ($p = 0.0027$) of the soil fauna (Figure S3).

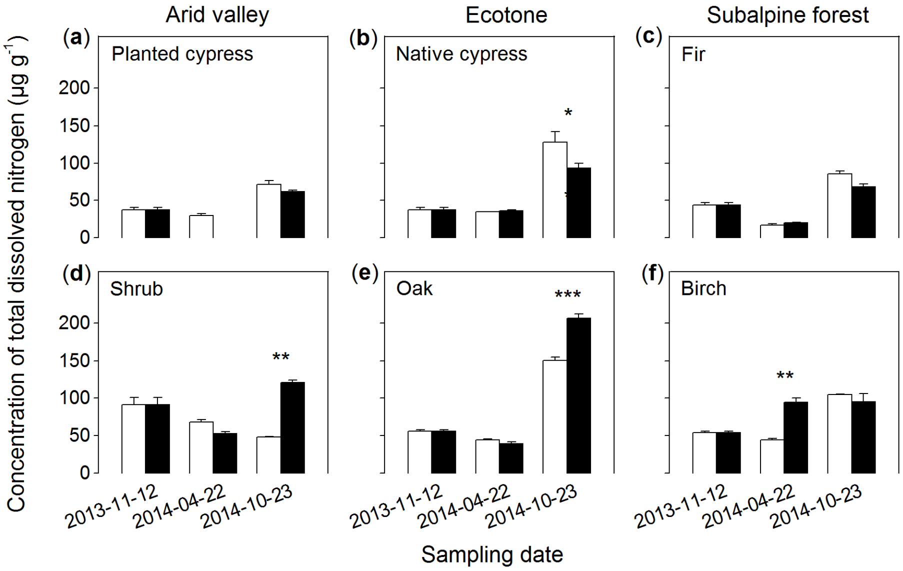

Figure 6. Concentrations (±SE, $n = 3$) of total dissolved nitrogen in the foliar litter with (white) and without (black) soil fauna. (**a**) planted cypress; (**b**) native cypress; (**c**) fir; (**d**) shrub; (**e**) oak; (**f**) birch. The designated samples of planted cypress litter on April 22, 2014, were lost due to strong winds; thus, these data are missing. Values with *, ** and *** are significantly different between the treatments at the $p = 0.05$, 0.01 and 0.001 levels, respectively.

4. Discussion

Litter decomposition is one of the fundamental life-supporting processes in terrestrial ecosystems [19], but there is still a considerable lack of knowledge on some general patterns regarding the potential interactions among the factors that limit litter decay [40]. Conceptual deductions have elucidated that the decomposition rate is hierarchically controlled by climate > litter quality > soil decomposer [41], whereas it is difficult to distinguish these factors separately [42,43]. García-Palacios et al. synthesized global data in faunal exclusion studies and showed that soil fauna stimulate decomposition rate by 37% at the global scale, but this effect varies greatly between ecosystems that present different temperature and moisture regimes [12]. In this study, we evaluated the effects of soil fauna on dissolved carbon and nitrogen in foliar litter along an altitudinal gradient with changes in climate and vegetation type and found that soil fauna decreased the concentrations of DOC and TDN but the effect varied between habitats and different types of litter with different qualities.

Freshly shed litter that has fallen on the soil surface provides an important source of shelter and a food resource for soil fauna [44], but low temperature is an important factor that limits faunal activity [21]. However, water content is another decisive factor in determining the abundance and activity of soil decomposers [45,46]; thus, it is unclear which factor dominates the faunal effect. In this study, with the increase in altitude from the arid valley to the subalpine forest, the temperature remarkably decreased, resulting in frequent soil freezing and thawing [38,47] (Table 1, Table S2). In contrast, the amount of rainfall and snowfall increased during the winter [48]. Our results showed that the faunal effect on DOC and TDN was greater in the subalpine forest during the winter and greater in the arid valley during the growing season (Figures 4 and 5). Such an effect of soil fauna corresponded with the water content in the foliar litter (Figure 6, Figure S1), implying that the temporal variations of the individual density and community abundance of soil fauna in foliar litter are related to precipitation [49] and not only temperature (Table 2, Table S5).

Winter is often referred to as the dormant season with low decomposer activity [50]; however, our previous studies conducted in the high-altitude subalpine forest indicated that soil animals were considerably abundant during the cold winter [21] and contribute to a litter decomposition rate of 22%–27% for fir and birch litter [51]. One important reason for this result was the presence of deep snow cover (approximately 20–30 cm extending from October to April of the following year [30]) in these forests, which allows soil decomposers to maintain considerable activity beneath the insulating snow cover [52,53]. Alternatively, snowmelt may directly contribute to the release of dissolved substances from foliar litter and thus indirectly provide a source of available carbon and nutrients for soil fauna [4]. Therefore, soil fauna exert a more complex influence than snow cover and its associated hydrological processes, and the effects of low temperature on DOC and TDN are not the most limiting effects in the subalpine forest. In contrast, in the arid valley, the upper soil underwent frequent freezing and thawing (Table S2), and no soil invertebrates were observed during the winter (Table 2). However, sufficient water accompanied by precipitation events during the growing season compensated for the faunal effect [49] by stimulating the release of DOC and TDN (Figures 4 and 5). This result also demonstrates the results observed in the subalpine forest.

Consistent with our previous investigations [21], this study indicated that oribatid mites (i.e., Prostigmata and Oribatida) and Collembola (i.e., Isotomidae) were the dominant communities of soil fauna at the studied sites (Table S3). Researchers found that oribatid mites are one of the most abundant soil meso-fauna in terrestrial ecosystems [54] and can increase microbial activity [55]. However, some fungivorous fauna in turn feed on fungi [56] because they cannot directly utilize resistant litter as a nutrient source [7]. On the other hand, studies have demonstrated that some oribatid mites can stimulate the release of dissolved carbon and nitrogen from fresh litter [55]. Thus, a central question involved in the faunal effect is the quality of the litter substrate. In this study, we selected two dominant species in each habitat with contrasting C/N ratios (Table S1) and found that the faunal effects on the release of both DOC and TDN from the foliar litter were greater for the high-quality litter (Table 4). When combined with all litter species, the C/N ratio was significantly (both $p < 0.001$) correlated with both the concentrations of DOC and TDN (Figure S2), which supports our second hypothesis.

High-quality plant litter is expected to stimulate Collembolan growth [23]. Our results also indicated that higher individual numbers (Table 2) and Simpson indexes of soil fauna (Table S5) were observed for birch litter, which had a lower C/N ratio than fir litter. However, more oribatid mites, such as Prostigmata (73%) and Oribatida (61%), were observed for the leaf litter (i.e., oak and birch). These fungivorous fauna were assessed to accelerate carbon and nitrogen mineralization by nearly 20% and therefore promote the release of dissolved carbon and nitrogen [55]. Thus, the different effects of soil fauna on DOC and TDN between high- and low-quality litter may be related to the feeding activity of soil animals.

5. Conclusions

This study highlighted two key unknowns regarding the effects of soil fauna on dissolved carbon and nitrogen in foliar litter during early litter decomposition. Current studies do not distinguish between the roles of temperature and moisture in modulating the effects of soil fauna [12]. We conducted a litterbag experiment in three diverse habitats and found that water, rather than temperature, impacts the effects of fauna on carbon and nitrogen in fresh litter. Moreover, this faunal effect is greater for high-quality litter. Although extrapolation of the local results is urgently needed, this information is projected to be important for obtaining a better mechanistic understanding of the role of soil fauna in ecosystem functions.

Supplementary Materials: The following are available online at www.mdpi.com/1999-4907/7/10/249/s1, Figure S1: Water contents (±SE, $n = 3$) in the foliar litter with (white) and without (black) soil fauna at different sampling dates, Figure S2: Concentrations of (**a**,**b**) dissolved organic carbon and (**c**,**d**) total dissolved nitrogen plotted against the carbon and nitrogen contents and the C/N ratios, respectively, Figure S3: Concentrations of (**a**–**e**) dissolved organic carbon and (**f**–**j**) total dissolved nitrogen of the foliar litter plotted against the individual number and Shannon-Wiener, Simpson, Piolou and Margalef indexes of soil fauna, Table S1: Initial chemical composition of the foliar litter (concentration ±SE, $n = 3$), Table S2: Mean litter surface temperature and frequency of freeze-thaw cycles at the experimental sites at different time periods, Table S3: Number of individuals (±SE, $n = 3$) and percentage (%) of soil fauna observed in the litterbag with 3 mm mesh size during the one-year decomposition period, Table S4: Levels of significance from the two-way ANOVA for comparing the effects of season, litter species and their interaction on the group number, individual number and Shannon-Wiener, Simpson, Pielou and Margalef indexes of soil fauna, Table S5: Shannon-Wiener, Simpson, Pielou and Margalef indexes (±SE, $n = 3$) of soil fauna observed in the litterbag with 3 mm mesh size, Data S1: Daily mean litter surface temperatures at the experimental sites, Data S2: Mass remaining of the foliar litter with and without soil fauna, Data S3: Concentrations of dissolved organic carbon and total dissolved nitrogen in the foliar litter with and without soil fauna, Data S4: Water contents of the foliar litter, Data S5: Initial chemical composition of the foliar litter, Data S6: Soil fauna observed in the litterbag with 3 mm mesh size.

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