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The Responses of Leaf Litter Calcium, Magnesium, and Manganese Dynamics to Simulated Nitrogen Deposition and Reduced Precipitation Vary with Different Decomposition Stages

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Abstract: Litter decomposition is a vital link between material circulation and energy flow in forest ecosystems and is intensely affected by global change factors, such as increased nitrogen (N) deposition and altered precipitation regimes. As essential nutrients, calcium (Ca), magnesium (Mg), and manganese (Mn) play crucial roles in plant energy metabolism, photosynthesis, and membrane transport of plants, and the major source of these nutrients is litter decomposition. However, the dynamics of Ca, Mg, and Mn during decomposition have been largely ignored. Thus, to better understand Ca, Mg, and Mn dynamics during leaf litter decomposition in the scenario of increasing N deposition and decreasing precipitation, we carried out a two-year field litterbag experiment in a natural evergreen broad-leaved forest in the central area of the rainy area of Western China. Two levels of N deposition (ambient N deposition and 150 kg·N·ha^{-1}·y^{-1}) and precipitation reduction (no throughfall reduction and 10% throughfall reduction) were set, i.e., control (Ctr: without nitrogen deposition or throughfall reduction), N deposition (N, 150 kg·N·ha^{-1}·y^{-1}), throughfall reduction (T, 10% throughfall reduction), and N deposition and throughfall reduction (NT, 150 kg·N·ha^{-1}·y^{-1} and 10% throughfall reduction). We found that leaf litter Ca concentration increased in the early decomposition stage and then decreased, while Mg and Mn concentrations generally decreased during the whole period of decomposition. The amount of Ca showed an accumulation pattern, while Mg and Mn generally showed a release pattern. N deposition and throughfall reduction affected the Ca, Mg, and Mn dynamics, varying with different decomposition stages; i.e., N deposition significantly affected the concentration and amount of Ca, regardless of the decomposition stages, while throughfall reduction significantly affected the Ca concentration in the whole and early decomposition stages. N deposition significantly affected the concentration and amount of Mg in the whole and early decomposition stages, while throughfall reduction had no significant effects. Throughfall reduction significantly affected the concentration and amount of Mn in the whole and late decomposition stages, while N deposition had no significant effects. Ca concentration generally showed a significant positive linear relationship with mass loss in the early decomposition stage; Mg concentration showed a significant positive linear relationship with mass loss in the Ctr and N treatments in the early and late decomposition stages; Mn generally showed a significant negative linear relationship with mass loss, regardless of the decomposition stage. Overall, the results suggest that Ca accumulation is more likely affected by N deposition, while Mg and Mn releases are more likely affected by N deposition combined with throughfall reduction, particularly in the early decomposition stage.

Keywords: global change; acid deposition; drought; mineral nutrient; micronutrients

1. Introduction

Since the Industrial Revolution, the concentration of reactive nitrogen (N) in the atmosphere has increased rapidly due to human activities, such as the burning of fossil fuels and the production and use of fertilizers, and has been continuously subsiding in terrestrial and aquatic ecosystems [1–3]. It is indisputable that global atmospheric N deposition has increased from 16 to 210 Tg $N{\cdot}y^{-1}$ [2,4]. In the last two decades, China has become the largest emitter of N deposition in the atmosphere worldwide [4,5]. The average total N deposition by China has been estimated to be 20.4 ± 2.6 $kgN{\cdot}ha^{-1}{\cdot}y^{-1}$ during the period of 2011 to 2015 [1]; in particular, in some tropical and subtropical forests, it has reached 30 to 73 $kg{\cdot}N{\cdot}hm^{-2}{\cdot}y^{-1}$ [6]. Meanwhile, with the increasing global temperature, global precipitation patterns have undergone great changes [7–9]. Human influence has contributed to the pattern of observed precipitation changes, i.e., the amount, timing, and distribution of precipitation have been changing worldwide since the mid-20th century [10]. Precipitation is projected to increase over high latitudes, the equatorial Pacific, and parts of monsoon regions, but decrease over parts of the subtropics, engendering and exacerbating regional drought conditions, especially in mid-latitude regions [10,11]. Increasing evidence shows that rapidly increasing N deposition and decreasing precipitation (often resulting in drought) can affect plant growth [12–14], change functioning and biodiversity [3,9,15,16], and impact key processes, such as litter decomposition [17–20], in terrestrial ecosystems at an unprecedented speed.

Reflecting the vital importance of decomposition in the C cycle and in energy and nutrient transfer in decomposer food webs, many studies have focused on rates and mechanisms of litter decomposition [21–23]. At large scales, such as the biome or global scale, variations in climate are shown to be key drivers of litter decomposition [21,24–26], while at the regional scale, recent studies suggest that litter characteristics affect the decomposition more strongly than climatic variables [27–29]. More and more studies have recognized that mineral nutrients, such as calcium (Ca), magnesium (Mg), and manganese (Mn), can be more important in driving litter decomposition than those more commonly used, such as C, N, or lignin, due to their vital roles in regulating decomposer and enzyme activities [27,28,30–32]. For example, positive correlations between initial Ca concentration and litter decomposition have also been reported previously, where Ca stimulation was proposed to act indirectly via soil pH or by directly stimulating earthworm activity [33,34]. Previous studies suggested that high Mg concentrations support microbial activity and enhance leaf litter mass loss [27,31]. Likewise, the importance of Mn for litter decomposition, especially in the late decomposition stage, has been significantly highlighted in recent studies, as Mn is a key component of the lignin-degrading enzyme manganese peroxidase (MnP), produced by white-rot fungi [31,35]. Moreover, in addition to studying the rates and mechanisms of litter decomposition, most studies have focused on the release of C and key nutrients, such as N and phosphorus (P), from decomposing litter, the dynamics of mineral nutrients have been largely ignored [33,35–38]. Significant knowledge gaps and uncertainties remain regarding the dynamics of mineral nutrients such as Ca, Mg, and Mn in decomposing litter, particularly under the background of rapid global changes.

To better understand the Ca, Mg, and Mn dynamics during leaf litter decomposition in the scenario of increasing N deposition and decreasing precipitation, we carried out a two-year field litterbag experiment in a natural evergreen broad-leaved forest in the central area of the rainy area of Western China. We simulated N deposition at a level of 150 kg·N·ha^{-1}·y^{-1} and simulated precipitation reduction in a 10% reduction of annual throughfall experiments by sheltering the forest floor area. Effects on the dynamics of concentrations and the amounts of leaf litter Ca, Mg, and Mn were studied. We hypothesized that (1) Ca, Mg, and Mn would be released as the decomposition time increased. Based on the previous findings that both N deposition [17,39,40] and reduced precipitation [18] retarded the decomposition process at our study site, we hypothesized that (2) N deposition and reduced precipitation would also suppress Ca, Mg, and Mn release. In consideration of the varying nutrient dynamics in different decomposition stages [24,26], we also hypothesized that (3) the responses of Ca, Mg, and Mg dynamics to N deposition and reduced precipitation would vary among the different decomposition stages.

2. Materials and Methods

2.1. Study Sites

Field experiments were conducted in a natural evergreen broad-leaved forest (102°59′ E, 30°03′ N, 1170 m a.s.l.) within the Bi Feng Gorge Scenic Spot, which is at the western edge of the Sichuan Basin, Southwestern China. The region is located in a subtropical moist forest zone that experiences a monsoon climate [17,18]. There is abundant rainfall along the western edge of the Sichuan Basin, known as the "rainy area of Western China" [17,41,42]. The mean annual precipitation is about 1700 mm and the mean annual air temperature is 16.2 °C with an average daily temperature ranging from 6.1 (January) to 25.4 °C (July). The dominant species in our study forest are *Schima superba*, *Lithocarpus hancei*, *Machilus pingii*, and *Pittosporum tobira*. There are few shrubs and herbs in the understory of the stands. The soil is classified as a Lithic Dystrudepts (according to the USDA Soil Taxonomy), derived from purple sandstone and shale, with a depth of more than 60 cm. The stand factor information and soil properties (0–20 cm) of the experimental site in November 2013 can be seen in Table S1.

2.2. Experimental Design

In January 2016, 12 plots (5 × 5 m each) at intervals of 5 m were randomly established in a representative natural evergreen broad-leaved forest at the Bi Feng Gorge Scenic Spot. The annual atmospheric deposition of wet N in the central area of the rainy area of Western China was 95 kg·N·ha^{-1} during the period of 2008 to 2010, and continues to increase [42,43]. Meanwhile, the annual precipitation of Ya'an City, located in the central area of the rainy area of Western China, decreased markedly over the last 20 years [18,44]. Thus, two levels of N deposition (ambient N deposition and 150 kg·N·ha^{-1}·y^{-1}) and precipitation reduction (no throughfall reduction and 10% throughfall reduction) were set in our study. We tested four treatments, i.e., control (Ctr: without nitrogen deposition or throughfall reduction), N deposition (N, 150 kg·N·ha^{-1}·yr^{-1}), throughfall reduction (T, 10% throughfall reduction), and N deposition and throughfall reduction (NT, 150 kg·N·ha^{-1}·y^{-1} and 10% throughfall reduction), with three replicates per treatments. Ammonium nitrate (NH_4NO_3) was used to simulate N deposition in our study, as the main forms of atmospheric N deposition in our study area are ammonium N and nitrate N [17,39,42,43]. We applied NH_4NO_3 twice a month, from March 2016 to March 2018. During each supply application, 44.64 g NH_4NO_3 was weighed for N and NT treatment and dissolved in 2 L of water and then sprayed evenly back and forth at a height of 50 cm above the ground for each plot. Meanwhile, 2 L of water without NH_4NO_3 was applied in the control and T treatment plots. We estimated that approximately 0.192 mm of simulated precipitation per year was added to the ground in each plot during the study period compared to the mean annual precipitation (1700 mm), which was negligible. Reduced precipitation was achieved by building a throughfall reduction device under the canopy, in reference to the guidelines for designing field experiments simulating precipitation extremes in forest ecosystems [8,18]. Briefly, a 6 m × 6 m roof with a rain gutter covered with ten translucent V-shaped PVC sheets (6 m × 0.05 m; covering 10% of the plot area) mounted to wood frames was installed above each throughfall reduction plot, more details of the device were described by Zhou et al. [18].

2.3. Leaf Litter Manipulation

In November 2015, when the peak of litter production occurred (October to December), we collected newly shed foliar litter from the studied forest by suspended litter traps. Upon arrival at the laboratory, foliar litter was air-dried and mixed evenly. Ten, 10.0-g air-dried samples were dried to a constant weight at 65 °C to determine water content and initial litter quality (see Table S2). Then, we calculated an index of the weight difference between the air-dried and oven-dried litter. Fifteen grams of oven-dried litter, calculated using the index of weight difference, were placed in nylon-mesh litterbags (20 cm × 20 cm). The surface layer of the litterbag was 3.00 mm to allow entrance to the decomposers, and the mesh size of the ground layer was 0.05 mm to prevent litter loss through the mesh net. In March 2016, litterbags were evenly arranged on the soil surface of the 12 plots. Altogether, we constructed 432 litterbags (12 sampling times × 3 bags per sampling time × 12 plots) in the field. To monitor the temperature in the litterbag during the decomposition process, we placed an iButton logger (DS1922E, Maxim Integrated Products Inc., Sunnyvale CA, USA) in a randomly selected litterbag for each treatment.

Every two months after the first simulated N deposition, three litterbags were collected per plot, i.e., in mid-May, July, September, and November 2016, mid-January, March, May, July, September, and November 2017, as well as mid-January and March 2018 (12 sampling times). When sampling, litter bags were placed in sealed and breathable black cloth bags at a low temperature and were then transported to the laboratory. After retrieval, the litter was removed from the litterbag, and were cleaned of soil and debris quickly. Then, the remaining litter in each bag was weighed and oven-dried at 65 °C until a constant weight was reached. After drying, each bag was reweighed individually to measure the mass loss and water content. Then, the litter was milled (<0.15 mm) and analyzed for Ca, Mg, and Mn concentrations.

The concentrations of Ca, Mg, and Mn were measured using an atomic absorption spectrometer (novAA400P, Analytik Jena GmbH, Jena Germany). Concentrations were expressed per unit of oven-dried sample (65 °C), all analyses were conducted in triplicate.

2.4. Calculations and Statistical Analysis

Total Ca, Mg, and Mn losses (%) were calculated based on the following equations [18,25,36]:

$$Ca\ loss\ (\%) = (X_0 \times Ca_0 - X_t \times Ca_t)/(X_0 \times Ca_0) \times 100 \quad (1)$$

$$Mg\ loss\ (\%) = (X_0 \times Mg_0 - X_t \times Mg_t)/(X_0 \times Mg_0) \times 100 \quad (2)$$

$$Mn\ loss\ (\%) = (X_0 \times Mn_0 - X_t \times Mn_t)/(X_0 \times Mn_0) \times 100 \quad (3)$$

where X_0 is the initial litter dry mass, X_t is the dry mass at sampling time t (year), Ca_0, Mg_0, and Mn_0 are the initial concentrations of Ca, Mg, and Mn (g·kg^{-1}), respectively. Ca_t, Mg_t, and Mn_t are the concentrations at sampling time t (year).

Generally, the decomposition of litter can be divided into three stages, based on the mass loss, i.e., early stage (0 < mass loss < 40%), late stage (40% < mass loss < 70%), and the final stage (mass loss > 70%), and the nutrient dynamics during the decomposition are different in different stages [22,24,45]. In our study, after 10 months' decomposition, the mass loss of all treatments was almost 40%, and at the end of experiment it was slightly less than 60% (mass loss < 70%; Figure S1). Thus, we separated our decomposition process into two stages, i.e., early stage (0–10 months) and late stage (10–24 months). To identify the effects N deposition and throughfall reduction on the dynamics of Ca, Mg, and Mn in the early, late, and whole (0–24 months, combine early and late stage) decomposition stages, we use linear mixed effect models with the restricted maximum likelihood estimation method [18] to inspect the effects of N deposition, throughfall reduction, sampling time, and their interactions on the concentrations and amounts of Ca, Mg, and Mn in different decomposition stages. N deposition, throughfall reduction sampling time, and their interactions were considered as fixed factors, while plot number was considered as a random factor in the models. Results were considered significant at $p < 0.05$. All statistical analyses were carried out using SPSS 27.0 (SPSS Inc., Armonk, NY, USA) for Microsoft Windows.

3. Results

3.1. Litter Temperature and Water Content during Decomposition

Litter temperature (day average temperature) followed a strong seasonal pattern during the study period of two years (Figure A1A in Appendix A), ranging from 0.2 (January 2017) to 21.5 °C (July 2016), while litter water contents varied slightly with the season (Figure A2A in Appendix A), ranging from 57.0% (May 2018) to 77.3% (September 2017). No significant differences in litter temperature were observed between treatments during the study period (Figure A1B in Appendix A), while T and NT treatments significantly decreased leaf water content compared to Ctr and N treatments ($p < 0.05$; Figure A2B in Appendix A).

3.2. Ca, Mg, and Mn Concentrations during Decomposition

Leaf litter Ca concentration increased during the first 10 months from an initial 1.59 ± 0.07 to 4.27 ± 0.22 g·kg^{-1}, and then decreased to 2.23 ± 0.25 g·kg^{-1} at the end of the experiment (Figure 1A). N deposition significantly affected the Ca concentration, regardless of the decomposition stages (whole, early and late decomposition stage), while throughfall reduction significantly affected the Ca concentration in the whole and early decomposition stages ($p < 0.05$; Table 1). On average, in the whole and early decomposition stages, the Ca concentrations were higher in N, T, and NT treatments, as compared to Ctr ($p < 0.05$); in the late decomposition stage, they were at a minimum in the NT treatment and at a maximum in Ctr ($p < 0.05$); however, differences were small as compared to N and T treatments (Figure S2A).

Leaf litter Mg concentration slightly decreased during the first four months from an initial 3.70 ± 0.41 to 2.47 ± 0.42 g·kg^{-1}. Then, it increased to 4.67 ± 0.07 g·kg^{-1} at the end of the experiment (Figure 1B). N deposition significantly affected the Mg concentration in the whole and early decomposition stages ($p < 0.05$), while throughfall reduction showed no significant effects (Table 1). On average, in the early decomposition stage, the Mg concentrations were at a minimum in the T treatment and at a maximum in the Ctr and NT treatments ($p < 0.05$); however, differences were small compared to the N treatment, while in the whole and late decomposition stages, there were no differences between treatments (Figure S2B).

Leaf litter Mn concentration strongly decreased during the first 10 months from an initial 0.53 ± 0.03 to 0.25 ± 0.02 g kg^{-1}, thereafter it decreased at lower rates to 0.14 ± 0.01 g kg^{-1} at the end of the experiment (Figure 1C). Throughfall reduction significantly affected the Mn concentration in the whole and late decomposition stages ($p < 0.05$), while N deposition had no significant effects (Table 1). In the whole decomposition stage, the Mn concentrations were at a minimum in the N treatment and at a maximum in the T and NT treatments; however, differences were small compared to Ctr ($p < 0.05$). In the early decomposition stage, the average Mn concentrations were higher in the N, T and NT treatments as compared to Ctr ($p < 0.05$). In the late decomposition stage, the average Mn concentrations were at a minimum in the N treatment and at a maximum in the T treatment; however, differences were small compared to Ctr and NT treatments ($p < 0.05$; Figure S2C).

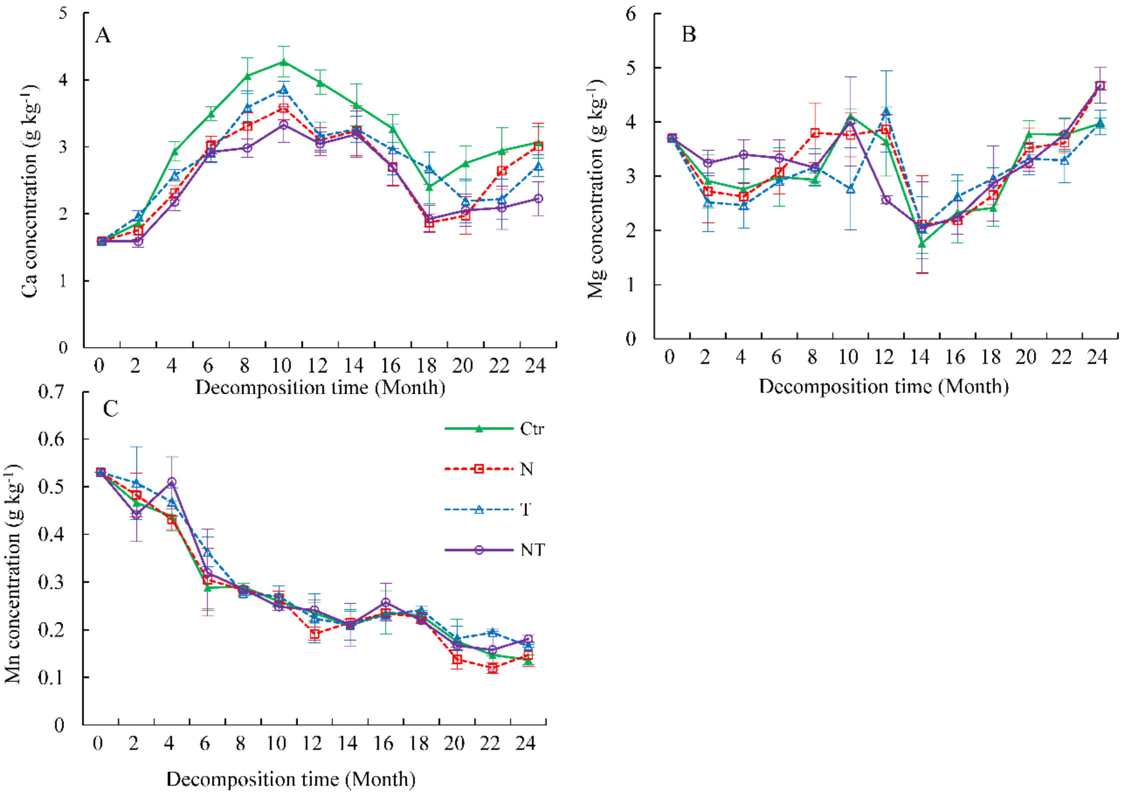

Figure 1. Changes in concentrations of Ca (**A**), Mg (**B**), and Mn (**C**) in leaf litter exposed in the field during the two-year experiment. Error bars represent standard deviations of the means (n = 3). Ctr: without nitrogen deposition or throughfall reduction, N: nitrogen deposition (150 kg·N·ha^{-1}·y^{-1}), T: throughfall reduction (10%), NT: nitrogen deposition (150 kg·N·ha^{-1}·y^{-1}) and throughfall reduction (10%).

Table 1. *F*- and *p*-values of the linear mixed effect models on the effects of N deposition, throughfall reduction, sampling time and their interactions on the concentrations of Ca, Mg, and Mn during different decomposition stages. Significant effects are given in bold.

Sources	**Whole Decomposition Stage (0–24 Months)**			**Early Decomposition Stage (0–10 Months)**			**Late Decomposition Stage (10–24 Months)**		
	d.f.	***F***	***p***	**d.f.**	***F***	***p***	**d.f.**	***F***	***p***
Ca concentration (g kg^{-1})									
Nitrogen deposition	**1**	**11.4**	**0.0096**	**1**	**28.4**	**0.0007**	**1**	**6.7**	**0.0322**
Throughfall reduction	**1**	**5.4**	**0.0490**	**1**	**10.0**	**0.0132**	1	3.9	0.0839
Sampling time	**11**	**531.6**	**<0.0001**	**4**	**1539.9**	**<0.0001**	**6**	**517.4**	**<0.0001**
Nitrogen deposition × Throughfall reduction	1	0.6	0.4629	1	0.8	0.4117	1	0.5	0.4853
Nitrogen deposition × Sampling time	**11**	**11.3**	**<0.0001**	**4**	**26.4**	**<0.0001**	**6**	**14.8**	**<0.0001**
Throughfall reduction × Sampling time	**11**	**18.5**	**<0.0001**	**4**	**13.2**	**<0.0001**	**6**	**45.0**	**<0.0001**
Nitrogen deposition × Throughfall reduction × Sampling time	**11**	**12.1**	**<0.0001**	**4**	**11.3**	**<0.0001**	**6**	**27.5**	**<0.0001**
Mg concentration (g kg^{-1})									
Nitrogen deposition	**1**	**4.2**	**0.0441**	**1**	**7.7**	**0.0244**	1	0.0	0.9633
Throughfall reduction	1	0.6	0.4346	1	0.4	0.5482	1	0.1	0.7139
Sampling time	**11**	**27.2**	**<0.0001**	**4**	**8.6**	**0.0001**	**6**	**46.7**	**<0.0001**
Nitrogen deposition × Throughfall reduction	1	0.4	0.5317	**1**	**5.5**	**0.0466**	1	1.9	0.2058
Nitrogen deposition × Sampling time	**11**	**2.2**	**0.0178**	4	0.1	0.9677	**6**	**3.1**	**0.0112**
Throughfall reduction × Sampling time	11	1.4	0.1991	4	1.8	0.1572	6	1.5	0.2144
Nitrogen deposition × Throughfall reduction × Sampling time	**11**	**3.6**	**0.0003**	**4**	**4.0**	**0.0099**	**6**	**2.7**	**0.0240**
Mn concentration (g kg^{-1})									
Nitrogen deposition	1	1.4	0.2397	1	0.3	0.6153	1	1.2	0.3088
Throughfall reduction	**1**	**10.2**	**0.0019**	1	2.7	0.1076	**1**	**6.8**	**0.0312**
Sampling time	**11**	**130.1**	**<0.0001**	**4**	**74.1**	**<0.0001**	**6**	**28.6**	**<0.0001**
Nitrogen deposition × Throughfall reduction	1	0.0	0.9183	1	0.8	0.3807	1	0.8	0.4099
Nitrogen deposition × Sampling time	11	0.7	0.7225	4	0.4	0.8256	6	1.7	0.1422
Throughfall reduction × Sampling time	11	1.5	0.1523	4	1.8	0.1469	6	1.5	0.1987
Nitrogen deposition × Throughfall reduction × Sampling time	11	1.2	0.3027	4	1.3	0.3020	6	1.0	0.4292

3.3. Ca, Mg, and Mn Releases during Decomposition

Leaf litter Ca loss decreased to −82.88 ± 4.86% during the first 16 months and then increased to 30.42 ± 4.61% at the end of the experiment, i.e., there was no net release of Ca from litter until shortly before the end of the experiment (Figure 2A). Ca accumulated during most of the decomposition process, showing an accumulation pattern in our study. N deposition significantly affected Ca loss, regardless of the decomposition stages ($p < 0.05$), while throughfall reduction had no significant effects (Table 2). On average, in the whole and early decomposition stages, the Ca losses were higher in the N and NT treatments, compared to the Ctr and T treatment ($p < 0.05$), i.e., the accumulation of Ca was significantly increased in N and NT treatments compared to the Ctr and T treatments; in the late decomposition stage, there were no differences between treatments (Figure S3A).

Leaf litter Mg loss increased to 74.77 ± 2.77% during the first 14 months and then decreased to 37.07 ± 3.77% at the end of the experiment (Figure 2B). Although there were some fluctuations, Mg generally showed a release pattern in our study. N deposition significantly affected Mg loss in the whole and early decomposition stages ($p < 0.05$), while throughfall reduction had no significant effects (Table 2). In the whole decomposition stage, the average Mg losses were at a minimum in the NT treatment and at a maximum in Ctr ($p < 0.05$); however, differences were small as compared to N and T treatments ($p < 0.05$). In the early decomposition stage, the average Mg losses were at a minimum in NT treatment and at a maximum in the Ctr and T treatments; however, differences were small as compared to N ($p < 0.05$). In the late decomposition stage, there were no differences in average Mg losses between treatments (Figure S3B).

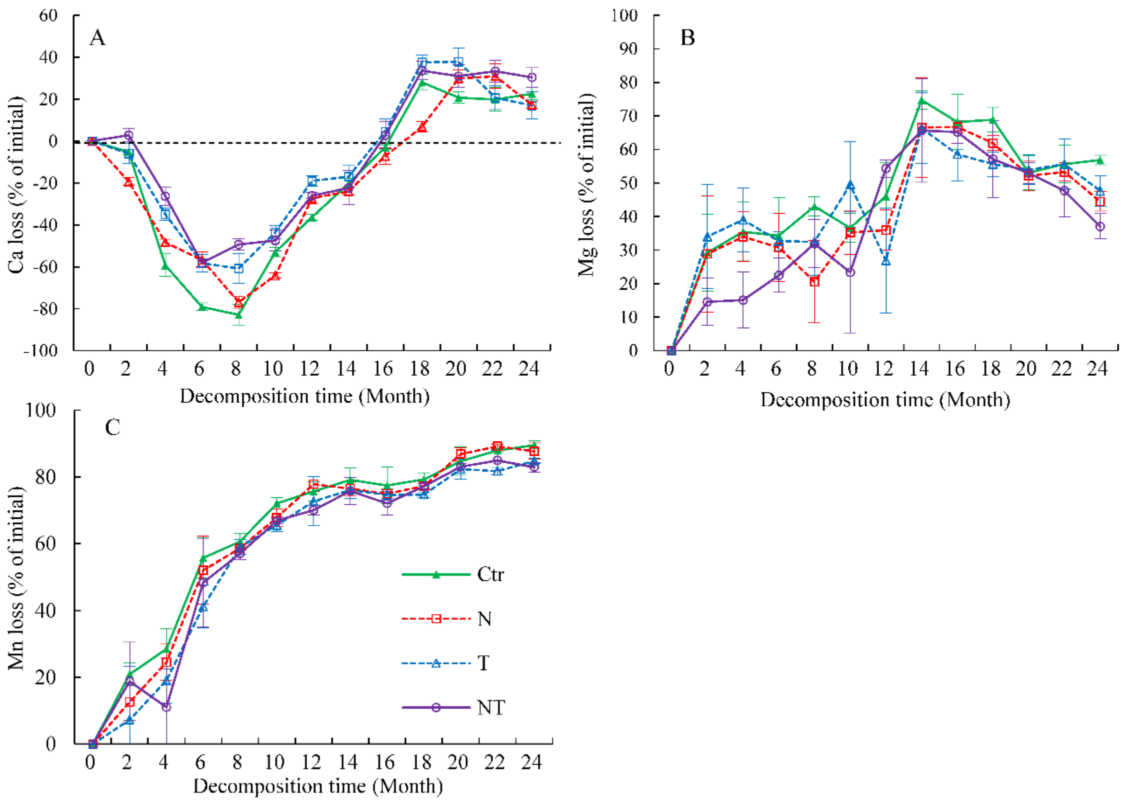

Figure 2. Changes in losses of Ca (**A**), Mg (**B**), and Mn (**C**) in leaf litter exposed in the field during the two-year experiment. Error bars represent the standard deviations of the means ($n = 3$). Abbreviations of treatments are given in Figure 1.

Table 2. *F*- and *p*-values of linear mixed effects models on the effects of nitrogen deposition, throughfall reduction, sampling time and their interactions on the loss of Ca, Mg, and Mn during different decomposition stages. Significant effects are given in bold.

Sources	Whole Decomposition Stage (0–24 Months)			Early Decomposition Stage (0–10 Months)			Late Decomposition Stage (10–24 Months)		
	d.f.	*F*	*p*	d.f.	*F*	*p*	d.f.	*F*	*p*
Ca loss (% of initial)									
Nitrogen deposition	**1**	**25.9**	**0.0009**	**1**	**69.8**	**<0.0001**	**1**	**10.0**	**0.0134**
Throughfall reduction	1	0.4	0.5272	1	4.0	0.0799	1	0.0	0.9038
Sampling time	**11**	**5549.2**	**<0.0001**	**4**	**3021.0**	**<0.0001**	**6**	**2234.9**	**<0.0001**
Nitrogen deposition × Throughfall reduction	1	0.1	0.7502	1	0.3	0.5837	1	0.0	0.8563
Nitrogen deposition × Sampling time	**11**	**59.8**	**<0.0001**	**4**	**66.5**	**<0.0001**	**6**	**31.0**	**<0.0001**
Throughfall reduction × Sampling time	**11**	**66.4**	**<0.0001**	**4**	**93.8**	**<0.0001**	**6**	**55.8**	**<0.0001**
Nitrogen deposition × Throughfall reduction × Sampling time	**11**	**54.3**	**<0.0001**	**4**	**87.6**	**<0.0001**	**6**	**46.9**	**<0.0001**
Mg (% of initial)									
Nitrogen deposition	**1**	**6.0**	**0.0400**	**1**	**11.2**	**0.0101**	1	0.5	0.4927
Throughfall reduction	1	2.8	0.1341	1	1.0	0.3415	1	2.6	0.1484
Sampling time	**11**	**38.3**	**<0.0001**	**4**	**1.5**	**0.2219**	**6**	**29.2**	**<0.0001**
Nitrogen deposition × Throughfall reduction	1	0.0	0.9070	1	2.4	0.157	1	2.4	0.1613
Nitrogen deposition × Sampling time	**11**	**2.1**	**0.0299**	4	0.2	0.9184	**6**	**3.0**	**0.0144**
Throughfall reduction × Sampling time	11	0.6	0.8495	4	0.4	0.7936	6	1.0	0.4111
Nitrogen deposition × Throughfall reduction × Sampling time	**11**	**3.7**	**0.0002**	**4**	**3.0**	**0.0335**	**6**	**3.6**	**0.0048**
Mn (% of initial)									
Nitrogen deposition	1	0.3	0.5729	1	0.3	0.5908	1	0.1	0.7410
Throughfall reduction	**1**	**15.5**	**0.0043**	**1**	**7.5**	**0.0252**	**1**	**16.1**	**0.0039**
Sampling time	**11**	**285.4**	**<0.0001**	**4**	**134.4**	**<0.0001**	**6**	**55.4**	**<0.0001**
Nitrogen deposition × Throughfall reduction	1	1.6	0.2474	1	2.3	0.1707	1	0.0	0.8556
Nitrogen deposition × Sampling time	11	0.7	0.7591	4	0.6	0.6533	6	1.5	0.2079
Throughfall reduction × Sampling time	11	1.1	0.3984	4	1.1	0.3963	6	1.0	0.4372
Nitrogen deposition × Throughfall reduction × Sampling time	11	1.4	0.1816	4	1.3	0.2802	6	1.1	0.3898

Leaf litter Mn loss strongly increased to 89.59 ± 1.19% during the whole experiment process (Figure 2B). Mn showed a net release pattern in our study. Throughfall reduction significantly affected Mn loss, regardless of the decomposition stages ($p < 0.05$), while N deposition had no significant effects (Table 2). In the whole decomposition stage, the average Mn losses were at a minimum in the NT treatment and at a maximum in Ctr ($p < 0.05$); however, differences were small as compared to N and T treatments. In the early decomposition stage, the average Mn losses were higher in the N, T and NT treatments, as compared to Ctr ($p < 0.05$). In the late decomposition stage, the average Mn losses were higher in the Ctr and N treatments, compared to T and NT treatments ($p < 0.05$; Figure S3C).

3.4. Relationships between Ca, Mg, and Mn Concentrations and Mass Loss

The concentration of Ca generally showed a significant quadratic relationship with litter mass loss in the whole decomposition stage (with the exception in Ctr treatment; Figure 3A), while with a significant positive linear relationship in the early decomposition stage (Figure S4A). In the late decomposition stage, only in the NT treatment Ca concentration and mass loss showed a significant negative correlation relationship (Figure S4B). The concentration of Mg showed a significant positive linear relationship with mass loss in the Ctr and N treatments in the early decomposition stage (Figure S4C), and a similar trend was also found in the Ctr, N, and T treatments in the late decomposition stage (Figure S4D). The concentration of Mn generally showed a significant negative linear relationship with mass loss, regardless of the decomposition stages (with the exception in NT treatment in the late decomposition stage; Figure 3C and Figure S4E,F).

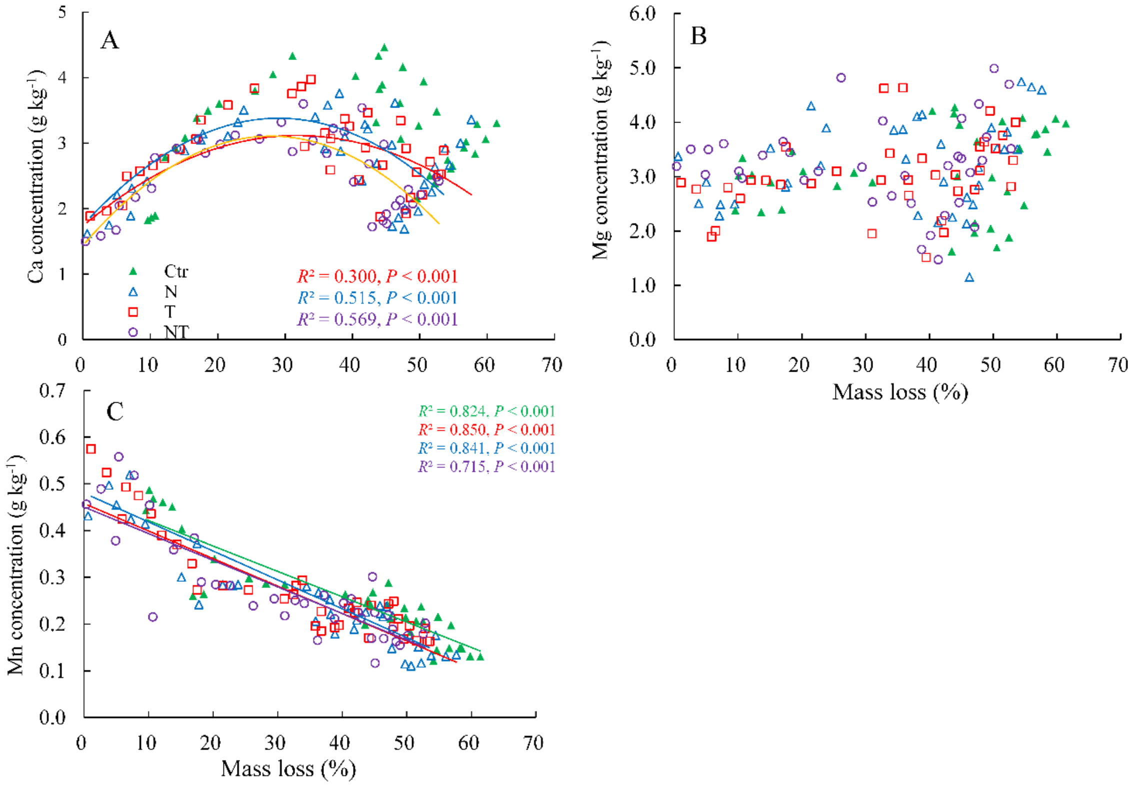

Figure 3. Relationships between Ca (**A**), Mg (**B**), and Mn (**C**) concentrations and mass loss in different treatments. Abbreviations of treatments are given in Figure 1.

4. Discussion and Conclusions

4.1. Ca, Mg, and Mn Dynamics during Decomposition

In our study, we found that Ca generally showed an accumulation pattern, which was contrary to our first hypothesis but in line with some previous studies [46–48]. Two explanations may account for the accumulation of Ca in our study. Firstly, Ca is always covalently bonded to pectin within the middle lamella in leaf litter [33]. Leaching or decomposition of more readily available organic components may cause a relative increase in Ca concentration, especially in the early decomposition stage [33,47]. In our study, the concentration of Ca generally showed a significant positive linear relationship with litter mass loss in the early decomposition stage, i.e., the rapid mass loss in the early decomposition stage may cause the accompanying accumulation of Ca, resulting in an increase in both the concentration and amount of Ca. Secondly, Ca is a main nutrient for living plants and for decomposers [33,34]. Thus, the accumulation of Ca in decomposition litters can be related to the organic decomposition rate and the demand for Ca in decomposer organisms [48,49], such as the formation of calcium oxalate by certain fungi [34]. Moreover, Ca can support the growth of white rot fungal species and is an essential cofactor of the lignin-degrading enzymes of decomposer microflora [50–52]. The Ca concentration and immobilization patterns observed in this study suggest that lignin degradation is already important in this early and late stages of decomposition, a net release pattern of lignin was observed in our previous paper [53].

Unlike Ca dynamics in the decomposing foliar litter, although with some fluctuations, Mg generally showed a release pattern in our study, which was in line with our first hypothesis and some previous studies [36,38,47,54]. Generally, as an easily leachable nutrient, Mg is an important constituent of chlorophyll; thus, it is easy to be leached out from plant litter in the early decomposition stage [36,38]. However, in the late decomposition stage, such a lack of an easily leachable nutrient may prevent or simply stop the microbial decomposition [38], which is why there was a relative increase in Mg concentration and amount during the period of 14–24 months in our study.

Likewise, we also found the amount of Mn decreased during the whole experiment process, showed a net release pattern, which was in line with our first hypothesis and some previous studies [50,55]. This may be related to the existence form of Mn in litter and the unique attributes of the elements. As Mn was found to be bound in organic complexes and occurred in ionic form in the litters [31,32], it is potentially more mobile than a mainly covalently bound nutrient, such as N, and the mobility may be dependent on pH [31]. Considering the low soil pH values in our study sites, Mn in the decomposing litters with highly mobile and show a net release pattern. However, some other studies found that Mn can accumulate in the litter due to the important role in manganese peroxidase formation, i.e., microbes can actively absorb Mn from ambient environment aiming for lignin degradation, especially in the late decomposition stage [31,35,36]. The Ca accumulated in the decomposing litter while Mn and lignin were released, suggesting that Ca may be more important in regulating lignin degradation as compared to Mn in our study.

4.2. Responses of Ca, Mg, and Mn to N Deposition and Reduced Precipitation

Contrary to our second and third hypotheses, we found N deposition significantly affected the Ca loss, regardless of the decomposition stages, while throughfall reduction showed no significant effects. As indicated by higher average amounts of Ca in litter in N deposition treatments (significant for N and NT treatments as compared to the Ctr and T treatments in the whole and early decomposition stages), N deposition facilitated the transfer of Ca into the litter, suggesting that N deposition aggravated Ca limitation, especially in early decomposition stages. The limitation of Ca would be dependent upon the availability of Ca in the soil. In the N deposition treatment, higher soil mineral N content would remove Ca from the exchange sites in the soil and stimulate biotic demands for Ca, thus resulting in increasing Ca availability in decomposition litters [56,57]. A ten-year N-addition experiment conducted in an N-rich tropical forest also found that added N reduced biologically available concentrations of Ca in the soil while it had no effects in the fresh foliar [56], the reduced Ca may potentially transport into the decomposing litters. However, in our previous studies performed in the same sites, we found N addition significantly decreased soil pH [58], microbial biomass C and N [58,59], and the soil arthropods during the litter decomposition [39,60]. The acidification caused by the N deposition resulted in suppression of decomposer activity [20,58,61,62], and less Ca will be transported. Due to the lack of knowledge of soil exchangeable cations and decomposer communities in this study, the mechanisms behind the Ca transfer facilitation, while having a negative effect on decomposers caused by the N addition need further investigation.

Partly in line with our second hypothesis, we found that N deposition significantly affected Mg loss while throughfall reduction significantly affected Mn loss. As indicated by the lower average amounts of Mg and Mn in litter in the NT treatment, N deposition combined with throughfall reduction significantly suppressed Mg and Mn release in the whole and early decomposition stages. This may be due to less leaching and a reduction in the activity and composition of decomposers in the scenario of increasing N deposition and decreasing precipitation. Many studies found that precipitation significantly enhanced litter decomposition, demonstrating faster litter decomposition rates in wet compared to dry areas [21,29,63]. Moreover, as mentioned above, we also found simulated N deposition significantly decreased the microbial biomass C and N and altered the structure and function of soil arthropods during the litter decomposition in our previous studies performed in the same sites [39,58–60].

5. Conclusions

Taken together, we found that Ca accumulated in the decomposing leaf litters while Mg and Mn release was observed in our study. Moreover, Ca accumulation is more likely to be affected by N deposition, while Mg and Mn release are more likely affected by N deposition combined with throughfall reduction, especially in the early decomposition stage. These results suggest that Ca may be more important, as compared to Mg and Mn, in regulating litter decomposition in the scenario of increasing N deposition and decreasing precipitation in our studied subtropical evergreen broad-leaved forest.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12111473/s1, Figure S1: Dynamics of accumulated mass loss of leaf litter in the litterbags in the field during the two-year experiment. Ctr: without nitrogen deposition or throughfall reduction, N: nitrogen deposition (150 kg·N·ha^{-1}·yr^{-1}), T: throughfall reduction (10%), NT: nitrogen deposition (150 kg·N·ha^{-1}·yr^{-1}) and throughfall reduction (10%). Figure S2: Effects of N deposition and throughfall reduction on mean Ca, Mg and Mn concentration of leaf litter exposed in the field during different decomposition stages based on the Linear mixed effects models. Different lowercase letters in the same decomposition stage denote significant differences (Multiple comparisons with Bonferroni adjustment method, $p < 0.05$) between treatments. Error bars represent the standard deviations of the means (n = 36). Abbreviations of treatments are given in Figure S1. Figure S3: Effects of N deposition and throughfall reduction on mean Ca, Mg and Mn loss of leaf litter exposed in the field during different decomposition stages based on the Linear mixed effects models. Different lowercase letters in the same decomposition stage denote significant differences (Multiple comparisons with Bonferroni adjustment method, $p < 0.05$) between treatments. Error bars represent the standard deviations of the means (n = 36). Abbreviations of treatments are given in Figure S1. Figure S4. Relationships between Ca, Mg, and Mn concentrations and mass loss in different treatments and different decomposition stages. A, B: the relationships between Ca concentration and mass loss in the early (A) and late (B) decomposition stage; C, D: the relationships between Mg concentration and mass loss in the early (C) and late (D) decomposition stage; E, F: the relationships between Mg concentration and mass loss in the early (E) and late (F) decomposition stage. Abbreviations of treatments are given in Figure S1. Table S1: Stand factor table of the experimental site and soil properties (0 ~ 20 cm) of the evergreen broad-leaved forest in November 2013. Table S2: Initial leaf litter quality in the broad-leaf litter of the experimental site.

Author Contributions: Conceptualization, S.Z. and C.H.; methodology, S.Z. and C.H.; formal analysis, S.Z. and L.X.; Software, S.Z., A.Z. and L.X.; investigation, G.Y., J.H., X.L., X.Z., L.T. (Liehua Tie), X.C., L.T. (Lihua Tu) and J.L.; data curation, S.Z., J.H., R.Y., Y.Y. and C.H.; writing—original draft preparation, S.Z., L.X. and C.H.; writing—review and editing, S.Z. and C.H.; visualization, S.Z.; supervision, C.H.; project administration, C.H.; funding acquisition, C.H. All authors have read and agreed to the published version of the manuscript.

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

Appendix A

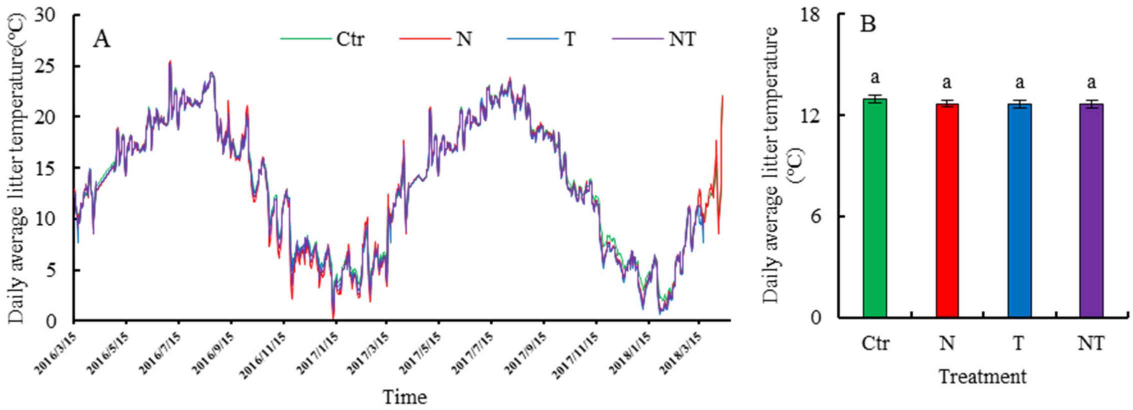

Figure A1. Dynamics of daily average temperature of leaf litter in the litterbags in the field during the two-year experiment from 15 March 2016 to 20 March 2018 (**A**). Different lowercase letters in panel (**B**) denote significant differences between treatments based on linear mixed-effects models (Multiple comparisons with Bonferroni adjustment method, $p < 0.05$). Ctr: without nitrogen deposition or throughfall reduction, N: nitrogen deposition (150 kg·N·ha^{-1}·y^{-1}), T: throughfall reduction (10%), NT: nitrogen deposition (150 kg·N·ha^{-1}·y^{-1}) and throughfall reduction (10%).

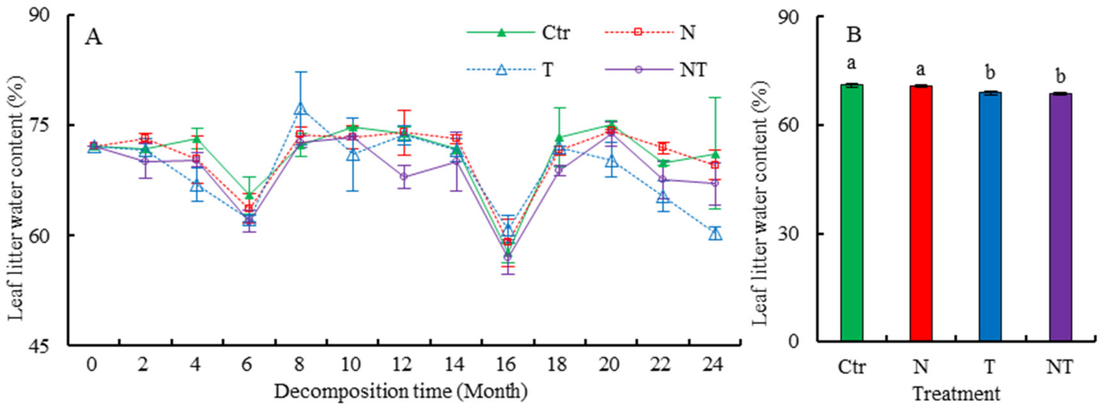

Figure A2. Changes in leaf litter water content exposed in the field during the two-year experiment (**A**). Different lowercase letters in panel (**B**) denote significant differences between treatments based on the linear mixed effects models (multiple comparisons with Bonferroni adjustment method, $p < 0.05$). Error bars represent the standard deviations of the means ($n = 36$). Abbreviations of treatments are given in Figure A1.

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