Effects of nitrogen and phosphorus addition on litter decomposition and soil enzyme activities with Salix cupularis in an alpine desert ecosystem

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Abstract

Nitrogen (N) and phosphorus (P) are important factors controlling biogeochemical cycling in terrestrial ecosystems and significantly affect the decomposition process of litter. However, N and P addition effects on litter decomposition, especially biological pathways in alpine grassland in northwest Sichuan, remain unclear. Therefore, this study explored the response mechanism of *Salix cupularis* litter decomposition and soil enzyme activity to different exogenous nitrogen and phosphorus additions and the inner relationship, combined with field in situ experiments and laboratory analysis methods. The results showed that: (1) In general, N, P and NP treatments significantly promoted the decomposition of litter, lignin and cellulose, and the addition of NP had a stronger effect in the same concentration level. P addition could alleviate the inhibitory effect of litter decomposition by high N concentration. (2) Litter C, N and P basically showed a sustained release pattern, and NP treatment had the strongest promoting effect. (3) Each treatment significantly increased the activities of soil invertase, cellulase, polyphenol oxidase, urease and phosphatase, and the NP treatment had the best effect. The rate of litter decomposition was significantly influenced by nutrient content as well as soil enzyme activity, where cellulose content and invertase activity may be a key factor controlling the rate of litter decomposition.

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Keywords: Salix cupularis; litter quality; decomposition rate; nitrogen and phosphorus additions; soil enzyme activities; alpine desert ecosystem

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1.Introduction

Litter plays an important role in maintains ecosystem productivity, enhancing soil fertility, improving soil texture, and optimizing the structure and metabolic characteristics of microbial communities in the system (Canessa et al., 2021, Huangfu and Wei, 2018). Litter decomposition is a crucial terrestrial ecosystem process which affects the nutrients cycling. (Chapin et al., 2002; Handa et al., 2014; Liu et al., 2016). Through material exchange and nutrient flow with soil, litter decomposition connects aboveground plants and soil and offers an essential way for plant-soil interaction (Chapin et al. 2002; Gregorich et al., 2016; Xiao et al., 2014). The changes of litter quality, including C, N, P concentration, cellulose and lignin can reflect the decomposition rate of litter matter to some extent (Yang et al., 2017). The decomposition rate is mainly controlled by climate, the plant properties, soil environment and microorganism activities, and most of these factors are affected by ecosystem nitrogen (N) deposition. (Van Diepen et al., 2015; Zhou et al., 2017; Liu and Greaver 2010). In addition, plant growth is usually limited by nitrogen or phosphorus, and is often even limited by both phosphorus concentration and effectiveness in the late stages of ecosystem regeneration, which will seriously affect the decomposition process of litter. (Vitousek et al. 2010; Jiang et al., 2016). Hence, understanding the effects of N and P input on litter decomposition is imperative.

The process of decomposition of litter by extracellular enzymes produced by soil microorganisms and plant roots is the key to promote biogeochemical cycling in terrestrial ecosystems (Yue et al., 2020). By changing soil physical and chemical properties, nitrogen and phosphorus can affect the effective use of soil nutrient by extracellular enzymes and regulate the nutrient uptake of plant, and ultimately affect the results of litter decomposition and feedback process (Song et al., 2014; Amend et al., 2015; Creamer et al., 2015; Waldrop et al. 2004). At the same time, litter decomposition is a continuous biodegradation process, the factors that dominate the mass loss of litter may change as the decay time of litter progresses (Huo et al., 2019). The addition of exogenous nutrients can usually accelerate the decomposition of litter in the early stage by providing nutrients necessary for microbial activities (Cuchietti et al., 2014), and slow the the decomposition of litter in later stage by change the microbial community and reduce enzyme activity (Cuchietti et al., 2014; Gill et al., 2021; Widdig et al., 2020). Therefore, it is essential to investigate the dynamic changes of litter decomposition and soil enzyme activity over time under N and P addition.

As a high altitude and unique type of grassland ecosystem in the world, alpine grasslands are extremely sensitive to global environmental changes and play an important role in regulating the global ecological environment (Chen et al., 2014). The alpine grassland of northwest Sichuan is located in the alpine semi-humid region on the southeastern edge of the Tibetan Plateau, with the world's largest plateau peat swamp wetlands, and its regional ecological and environmental status is very important (Hoover et al., 2016; Suseela et al., 2018). Over the years, due to the intensity of human activities and overgrazing, the alpine grasslands of northwest Sichuan have faced important ecological problems such as the degradation of grassland functions and severe desertification (Jiang et al., 2018). Grassland desertification control has become an important task for regional ecological restoration. Because of its cold tolerance and drought resistance, Salix cupularis can prevent cold winds from fixing sand, improve the alpine ecological environment, promote the growth of other herbaceous plants, and gradually form a stable shrub ecosystem in the ecological restoration process (Zhang et al., 2016; Yang et al., 2017). Therefore, we conducted a N and P addition experiment in Salix cupularis ecosystem in alpine sandy land in northwest Sichuan. We aim to reveal the response of Salix cupularis litter decomposition and soil enzyme activities to different exogenous nitrogen and phosphorus additions and their inner relationship, and provide scientific basis for the formulation of ecological restoration measures as well as natural resource management and regional sustainable development in northwest Sichuan.

2.Materials and methods

2.1. Study site

The study area is in Hongyuan County, Aba Tibetan and Qiang Autonomous Prefecture, northwest of Sichuan, China, on the southeastern edge of the Tibetan Plateau, and is the largest plateau peat swamp wetland area in the world, whose special ecological geographic location makes it an important regulator of the global ecological environment. The study was carried out in Wache Township, Hongyuan County (33°10'N, 102deg37'E), a typical demonstration area for sandy control in Sichuan Province. The sample area is flat, with a relatively uniform topography and slope. The topography slopes from southeast to northwest, and the landscape is typical of the transition from mountain plain to hilly plateau, with an altitude of 3600 m above sea level. The climate is continental plateau cold temperate monsoon climate. The mean annual precipitation is 791.95 mm and temperature is 1.5. The plants are dominated by sea buckthorn, Salix cupularis and medicinal plants, include Rhodiola rosea, Qianglong, Songbei, Astragalus, Cordyceps, Kobresia setchwanensis, Leymus secalinus, and Anaphalis flavescens. The area is fenced off from grazing and the experiment was conducted in an ecological restoration area of Salix cupularis.

2.2. Experimental design

In August 2018, an area of Salix cupularis with basically the same growth condition was selected to delineate a test sample site for exogenous N and P addition. The soil basic physicochemical properties, site conditions and initial values of the chemical composition of litter in the test plots are shown in Table 1. The topography and climate types in western China are complex, and the atmospheric N deposition is lower compared with other regions, with an annual N deposition of 3.62 g*m^{-2}*year^{-1} in western Sichuan (Yang et al., 2018). A According to the N deposition and N, P ecological stoichiometric ratio, control group without fertilization, three fertilization types (N, P, and NP) and three fertilization gradients were set up in this experiment for a total of 10 treatments: low N (N1, 3 g*m^{-2}*year^{-1}), medium N (N2, 6 g*m^{-2}*year^{-1}) and high N (N3, 9 g*m^{-2}*year^{-1}); low P (P1, 1 g*m^{-2}*year^{-1}), medium P (P2, 3 g*m^{-2}*year^{-1}), and high P (P3, 6 g*m^{-2}*year^{-1}); low NP (N1+P1), medium NP (NP2, N2+P2) and high NP (NP3, N3+P3). Urea ($CO(NH_2)2$, N: 46.67%) and sodium dihydrogen phosphate (NaH_2PO_4, P: 44.65%) were used as fertilizers. A completely randomized design was used to randomly select three Salix cupularis for each treatment in test plot, for a total of 30 Salix cupularis. A 1mx1m sample square was randomly laid within the vertical projection of the canopy of the thickets, and the litter was collected, removed from the thickets, and brought back to the laboratory for drying and shredding. A portion of the shredded litter was used for initial nutrient concentration analysis, and three samples (50g) of each treatment were accurately weighed, mixed with each addition treatment, and packed into 10cmx15cm nylon mesh decomposition bags. The nylon mesh bags were randomly placed in the 1mx1m sample square of the thickets, sealed with sealing clips to ensure that soil animals could not enter, and the four corners of each litter bag were fixed with stainless steel wire ties.

2.3. Soil sampling and laboratory analyses

The experiment was conducted on August 12, 2018 for in situ simulation of the litter decomposition of exogenous N and P addition, and the nylon net bags were retrieved on the 90th, 270th and 360th days of decomposition, respectively. After retrieval, the surface of the net bags was gently brushed, and plant fine roots and gravel were picked out from the bags, followed by washing the soil in the bags with ultrapure water. Each treatment (3 bags) was mixed well and dried in an oven at 60 for 72 h to a constant weight, then weighed and recorded after passing through a 1 mm steel sieve, and crushed through a 0.149 mm steel sieve for determination of total carbon, total nitrogen, total phosphorus, lignin and cellulose concentration of litter. At the same time, soil was collected from the 0-10 cm layer under the nylon net bags. Three bags of soil samples from each sample were mixed into one bag, totaling 30 bags.

Plant total carbon was determined by concentrated sulfuric acid-potassium dichromate oxidation method, total nitrogen by concentrated sulfuric acid-hydrogen peroxide-boiling-Kaeschner method, and total phosphorus by molybdenum antimony anti-colorimetric method (Lu, 1999); lignin and cellulose were determined by acid detergent fiber method (ADF method); soil organic carbon (SOC) was determined using potassium dichromate external heating method, total nitrogen (TN) by Kaeschner method, total phosphorus (TP) by molybdenum antimony anti-colorimetric method. 3,5-dinitrosalicylic acid colorimetric method for the determination of soil invertase (INV) and cellulase (CBH), sodium phenol-sodium hypochlorite colorimetric method for the determination of urease (UG), sodium hydrogen phosphate colorimetric method for the determination of alkaline phosphatase (AP), and o-phenylene triol colorimetric method for the determination of polyphenol oxidase (POX) (Blair et al., 1994).

2.4. Statistical analyses

The mass remaining of litter (M_r) (%) during the N and P addition test was calculated using the formula:

$M_r = M_t / M_0$?100% (1)

Where M_t and M_0 refer to the mass (g) of litter at time t of decomposition and the initial mass (g) of litter, respectively.

The value of the rate constant k for decomposition of litter matter was calculated by (Olson, 1963):

$M_t / M_0 = e^{-kt}$ (2)

Where k refers to the annual decomposition rate constant of litter.

The time to decompose 50% ($T_{0.5}$) and 95% ($T_{0.95}$) of the litter was calculated (Olson, 1963) as:

$T_{0.5} = -\ln (1 - 0.5)/k$ (3)

$T_{0.95} = -\ln (1 - 0.95)/k$ (4)

The lignin, cellulose, total carbon, total nitrogen and total phosphorus remaining rates (M_{cr}) (%) during decomposition of litter were calculated as:

$M_{cr} = (C_t$?$M_t) / (C_0$?$M_0)$?100% (5)

Where C_t and C_0 refer to the litter nutrient concentration (g*kg^{-1}) at time$t$ and the initial litter nutrient concentration (g*kg^{-1}), respectively.

The results of the study were processed and analyzed using SPSS 22.0 and R 3.6.3. One-way ANOVA was used to analyze the differences in the decomposition constants (k), the mass remaining of litter, lignin, cellulose, total carbon, total nitrogen and total phosphorus remaining rate, and soil enzyme activities under different treatments at the same decomposition time, and three-way repeated measures ANOVA (RM-ANOVA) was used to investigate the reciprocal effects of N and P addition for each index and their synergistic effects with decomposition time. Pearson correlation analysis and linear regression analysis were preformed to evaluate the correlation between the decomposition rate of litter, litter endogenous nutrient concentration and soil enzyme activity. To compare the relative importance of litter endogenous nutrient concentration and soil enzyme activity on K-value, the relative influence analysis was calculated using the "gbm" package.The results were plotted by Origin 2022 and R 3.6.3.

3.Results

3.1. Litter mass remaining and decomposition rate constant

The results showed that N, P and NP additions significantly contributed to the decomposition of litter matter (Fig.1). At 360 days, the mass remaining of litter was 53.62%, 62.36% and 65.16% in N1, N2 and N3 treatment; 55.56%, 53.02% and 51.35% in P1, P2 and P3 treatment; 51.05%, 47.72% and 49.23% in NP1, NP2 and NP3 treatment, respectively. One-way ANOVA showed that compared to CK, there were significant differences in mass remaining of litter in different concentrations of N, P and NP treatments, respectively

($P < 0.001$). Under N addition, N1, N2 and N3 treatments significantly reduced mass remaining in the early stages of decomposition, and only N1 treatment had a significant effect in the middle and late stages of decomposition; under P addition, P1, P2 and P3 significantly reduced mass remaining in each decomposition period; under NP addition, NP1, NP2 and NP3 treatments significantly reduced mass remaining in the middle and late stages of decomposition, and no significant difference was found between NP2 and NP3 treatments. The RM-ANOVA showed that the interaction of N and P additions and their synergistic effect with decomposition time had a significant effect on the mass remaining of litter ($P < 0.001$) (Table S1).

The litter decomposition rate constant (K) increased significantly with increasing P addition concentration with the highest value in the P3 treatment (0.67 year^{-1}), and showed a trend of increasing and then decreasing with increasing N and NP addition concentrations with the highest values in the N1 (0.62 year^{-1}) and NP2 (0.73 year^{-1}) treatments, respectively (Table 2). In the CK treatment, the time required for 50% ($T_{0.5}$) and 95% ($T_{0.95}$) of litter decomposition was 1.67 and 7.21 years, respectively. Under N addition, $T_{0.5}$ and $T_{0.95}$ were 1.11 and 4.81 years for the N1 treatment, 1.47 and 6.34 years for the N2 treatment, and 0.62 and 6.99 years for the N3 treatment, respectively. Under P addition, $T_{0.5}$and $T_{0.95}$ were 1.18 and 5.10 years for the P1 treatment, 1.09 and 4.72 years for the P2 treatment, and 4.49 and 1.04 years for the P3 treatment, respectively. Under NP addition, $T_{0.5}$and $T_{0.95}$ were 1.03 and 4.46 years for NP1 treatment, 0.94 and 4.05 years for NP2 treatment, and 0.98 and 4.12 years for NP3 treatment, respectively. The RM-ANOVA showed that the interaction of N and P additions and their synergistic effect with decomposition time had a significant effect on the decomposition rate constant ($P < 0.001$) (Table S1).

3.2. Litter lignin, cellulose and nutrient remaining rate

Overall, the lignin remaining rate showed a trend of decreasing and then increasing under N, P and NP addition, and the cellulose remaining rate continued to decrease with increasing decomposition time (Fig.2). Low concentrations of N, P and NP addition could promote the decomposition of lignin and cellulose, while high concentration treatment inhibited this effect, and lignin was accumulated under N3 treatment (105.61%). The one-way ANOVA showed that compared to CK, the lignin and cellulose remaining rates of N, P and NP treatments were significantly different ($P < 0.001$). After 360 days of decomposition, the lignin remaining rate was the lowest in N2 (68.46%), P1 (60.73%) and NP1 (57.84%) treatments, respectively, and the cellulose remaining rate in N1 (27.65%), P2 (29.72%) and NP1 (25.06%) treatments, respectively. It was noteworthy that the lignin remaining rate was significantly higher in both N3 and NP3 treatments than CK during litter decomposition ($P < 0.05$). The RM-ANOVA showed that the interaction of N and P addition and its synergistic effect with decomposition time had a significant effect on both lignin and cellulose remaining rates ($P < 0.001$) (Table S2).

The results showed that N, P and NP additions could significantly promote the release of litter carbon (C) compared to CK treatment (Fig.3). During the litter decomposition, the litter C remaining rate was not significantly different among different N addition concentrations, and the lowest in N3 treatment (38.30%) in late stage; in the late stage, the P3 (24.69%) and P2 (24.35%) treatments were significantly lower than P1 (26.63%); in the early and middle stages, NP3 treatment was significantly lower than other treatments, and in the late stage the NP2 (14.92%) treatment was significantly lower than other treatments. Compared with CK, N addition significantly inhibited the release of litter nitrogen (N) during the decomposition process, and the effect was strengthened with increasing N concentration and the highest N remaining rate (70.91%) was in N3 treatment; P addition significantly reduced the N remaining rate and the lowest value (25.06%) was in P3 treatment; NP2a and NP3 treatments significantly increased the N remaining rate in the early and middle stages, and NP1 was significantly lower than CK treatment with the lowest value of 34.29% in late stage. Compared with CK treatment, N addition significantly inhibited the release of litter phosphorus (P) in the early and late stages of decomposition, and the lowest P remaining rate (35.94%) was in N2 treatment; P3 addition significantly increased the litter P remaining rate in early and late decomposition, while P1 treatment significantly reduced the P remaining rate with the lowest value of 54.67% in the middle and late stages ; NP1 treatment significantly reduced the P remaining rate in the middle and late stages with the lowest value of 29.31%. The RM-ANOVA showed that the interaction of N and P addition and its synergistic effect with decomposition time had a significant effect on the litter C, N and P remaining rates ($P < 0.001$) (Table S2).

3.3. soil enzyme activity

The results showed that N, P and NP additions significantly increased the activities of INV, CBH, POX, UG and AP compared to CK treatment ($P<0.05$) (Fig.4). During the litter decomposition, the activities of INV, UG and AP decreased in the middle stage and increased in the late stage. The CBH activity was highest in 90 days of decomposition and then continued to decline, and the POX activity showed an increasing trend. In the late stage, with increasing N addition, both CBH and AP activities increased significantly, INV and POX activities decreased significantly, while UG showed a trend of first increase and then decrease. With the increase of P addition, the activities of INV, CBH and AP decreased significantly, and the activities of POX showed a trend of first increase and then decrease, while P addition had no significant effect on soil UG activity. With increasing NP addition, AP activity decreased significantly, INV and CBH activities showed a trend of first increase and then decrease, while NP addition had no significant effect on polyphenol oxidase and urease. The RM-ANVON showed that the interaction of N and P addition and its synergistic effect with decomposition time had significant effects on INV, CBH, POX, UG, and AP ($P < 0.001$) (Table S3).

3.4. Influencing factors in the decomposition of litter

The Pearson's correlation analyses and linear regression analysis showed that the litter K -value was positively correlated with cellulose content ($R^2 = 0.217$, $P < 0.001$), INV ($R^2 = 0.242$, $P < 0.001$), and CBH ($R^2 = 0.1$, $P < 0.01$) activity, and negatively correlated with N/P ($R^2 = 0.069$, $P< 0.05$) (Fig.5 and 7). Moreover, the contributions of litter cellulose content (28.53%) and soil INV activity (26.06%) to K were significantly higher than other indicators (Fig.6).

4.Discussion

4.1. Effects of nitrogen and phosphorus addition on litter decomposition rate

The input of N into the ecosystem changes processes such as microbial community structure and extracellular enzyme activity, which can affect the decomposition of litter matter (Hernandez et al., 2019; Wu et al., 2013). The mass remaining of litter in the early stage of decomposition was significantly lower than CK at low N concentration (Fig.1), indicating that the moderate N addition in the early stage was beneficial to litter decomposition, while too much was inhibitory, which is consistent with previous reports (Chen et al., 2013; Song et al 2015). Similarly, the low concentration N treatment had higher $k$values and was able to accelerate the decomposition of litter (Table 2). This is because moderate N input reduces the litter C/N ratio and adjusts the suitable conditions for litter decomposition, while high N input significantly reduces the soil pH, which is not conducive to microbial decomposition activities and tends to break the N/P balance and enhance ecosystem phosphorus limitation (Chen et al., 2013; Song et al 2015; Heuck et al., 2018). During the decomposition process, the high P concentration treatment had a larger k value and lower mass remaining of litter, indicating that P addition significantly stimulated litter decomposition, and the higher the concentration the stronger the stimulating effect. This could be explained by the fact that, on the one hand, P addition relieved the P nutrient limitation of ecosystem in the early stage of litter decomposition, and on the other hand, it increased the soil nutrient effectiveness, thus improving microbial activity as well as phosphorus use efficiency (Lin et al., 2019). The mass remaining rate of litter in all concentrations of NP treatment was lower than N and P treatment during the litter decomposition, indicating that NP addition had the strongest effect on litter decomposition. The results also indicated that N and P addition had an interactive effect on litter decomposition, and P addition may alleviate the inhibitory effect of high N on litter decomposition (Hao et al., 2013). In the study, increasing NP concentration slowed down the decomposition rate of litter, which may be related to the chemical regulation of phosphorus content in litter (Zheng et al., 2017). Overall, the decomposition rate of litter was higher in the early stage than in the middle and late stages, which could be because the study area is in highland alpine region, and rapid reproduction and growth of soil microorganisms would consume a large amount of nutrients and accelerate the litter decomposition in the early stage (July-October).

4.2. The dynamics of litter nutrient concentration during litter decomposition

Lignin, a recalcitrant component of litter, is difficult to be decomposed by various types of hydrolytic enzymes in soil due to its complex aromatic structure, and its degradation is an important process in the decomposition of plant litter. As a whole, in each treatment the lignin remaining rate was decreased faster in the early and middle stages than in late stage, which indicated that both N and P could significantly affect lignin degradation in the early and middle litter decomposition process (Fig.2), which was consistent with previous reports (Song et al., 2017; He et al., 2019). This may be because the main influencing factor of early lignin degradation is the initial nutrient content of litter, and the input of N and P increased the integrated level of initial effective nutrients and reduced the critical point of decomposition of lignin. (Song et al., 2017; He et al., 2019). The study showed that in the late stage P addition could accelerate the lignin decomposition, while the high concentration of N and NP addition would accumulated the lignin, which indicated that high concentration of N input inhibits lignin degradation and the alleviating effect of P input on the limitation of energy supply for soil microbial activity diminishes with increasing concentration of N input, and the high concentration of NP treatment reduces the synergistic effect of N and P for litter decomposition (Jiang et al., 2019). High concentrations of N input altered microbial habitat and community composition, inhibited lignin-related decomposition enzyme activities, and reduced microbial decomposition capacity (Rinkes et al., 2016; Gao et al., 2017). Plant cellulose is an important carbon source for soil microbial activity and significantly influenced by soil enzyme activity as a relatively abundant biopolymer in the ecosystem (Thomson et al., 2013). During the decomposition process, the cellulose remaining rate of litter showed a decreasing trend and the decrease of cellulose under NP treatment was higher than N and P treatment alone. This was due to the fact that N and P inputs reduced C/N and C/P ratios, increased microbial activity and cellulase activity, resulting in a decrease of cellulose content (Jiang et al., 2014; Talbot et al., 2012). The correlation and relative influence analysis showed that the cellulose content had a significant positive correlation with the litter decomposition rate, and its contribution to litter decomposition was the largest (Fig.5-7). This may be because cellulose is the most abundant polysaccharide compound in litter, and the exogenous nutrients addition change the community structure of decomposers, enabling them to decompose more cellulose to obtain energy materials (Štursová et al., 2012; Talbot and Treseder, 2012). At the same time, cellulose mainly require decomposition by cellulase and invertase (Xiao et al., 2018), thus cellulase and invertase activities were also significantly positively correlated with litter decomposition rate.

Litter nutrients are key factors affecting the composition of bacterial communities during decomposition, and C, N, and P stoichiometry significantly affect the structure and function of microorganisms, thus altering the fixation and mineralization processes of litter N and P (Barantal et al., 2014; Xu et al., 2020). The results showed that N, P and NP addition significantly promoted the degradation of carbon (C) in litter (Fig.3), because the nutrient input compensated for the environmental nutrient deficiency of microbial growth in the study area and accelerate the consuming of carbon sources by microorganisms (Ren et al., 2018). In the late stage of decomposition, the NP treatment had a significantly lower C remaining rate than the N and P additions alone, and had the highest contribution to the litter C release, which indicated that N and P additions had interactive effects on litter C release (Wang et al., 2017).However, the synergistic effect of high N and P addition might lead to microbial carbon utilization above the critical level, resulting in an increase of litter C remaining rate in the late stage of decomposition. In the study, N and P addition were able to accelerate the litter P and N release , while causing the accumulation of N and P, respectively, and the changes were aggravated with the increase of concentration. This is because, on the one hand, N and P addition reduced litter C/N and C/P, increased N and P hydrolase activity, which accelerated the decomposition of litter N and P (Jian et al., 2016); on the other hand, long-term N and P addition alone increased nutrient limitation in the ecosystem and produced nutrient enrichment with the increase of N and P input (Jie et al., 2014; Chen et al., 2012), while NP treatment could alleviate this nutrient limitation to some extent. During the decomposition process, the gradual decrease of litter N/P reflected that the study area was mainly N-limited and the litter N release rate of *Salix cupularis* after removing N, P limitation was higher than P.

4.3. The dynamics of soil enzyme activities during litter decomposition

Invertase and cellulase are produced by microorganism and play important roles in the decomposition of litter, and their activities are limited by the number and structural function of the microbial community, the chemical nature of the litter substrate, and the elemental stoichiometry and nutrient effectiveness (Wang et al., 2010).The results showed that in each nutrient addition invertase and cellulase activities were significantly increased with significant effect in the early stage of decomposition, which corresponded to the rapid degradation of polysaccharide compounds(e.g. cellulose) in the early stage (Fig.4). Cellulose is an easily decomposable substrate at the initial stage and N and P addition effectively stimulated the increase in carbon hydrolytic enzyme activities and the exudation of substrates for enzymes (Wang et al., 2010; Song et al., 2018). The decrease of INV activity with the increase of N addition concentration might be related to the inhibition of litter decomposition and reduction of the amount of substrate for INV action under high N concentration treatment (Ge 2017). In the late stage of decomposition, the CBH activity of NP treatment showed a decreasing trend. This may be due to the fact that in the early stage, the cellulose-like polysaccharide content of litter increased with the input of NP and the limitation of litter N and P was removed, while litter carbon gradually became the main influencing factor in the later stage, and thus cellulase activity was inhibited (Chen et al., 2014). POX activity is an important indicator of lignin degradation. In the study, POX activity decreased with the increase of N addition, which corresponded to the change of lignin. N input promoted the polymerization of lignin-like aromatic compounds with phenols into refractory substances and limited the activity of POX (Wang et al., 2019; Song et al., 2018). Meanwhile, POX activity was significantly higher in middle concentrations of P and NP addition than in low and high concentrations. This suggests that the moderate amount P input might disrupt this bio polymerization and alleviate P limitation (Kunito et al., 2009; Jiang et al., 2014). This was also verified by the results of the correlation analysis in this study, in which the rate of litter decomposition was negatively correlated with lignin/P and positively correlated with litter P content.

Soil UG and AP are important drivers of the carbon, nitrogen and phosphorus cycle in the ecosystem, and significantly affected by changes of litter decomposition conditions such as exogenous N, P addition (Zhang et al., 2017). Compared to CK, UG activity showed a significant increase in all treatments, while the effect was inhibited in high N addition (Fig.4), which was inconsistent with the previous study (Guan et al., 2019). This is because the substrate exudation rate was decreased by the slowing down of the degradation process of litter under high N treatment, resulting in lower UG activity (Zeng et al., 2019). However, there was no significant difference between different concentrations of P, NP additions, which might be due to the fact that the UG on topsoil was more sensitive to N addition than P addition (Jiang et al., 2014). In the study, AP activity was increased with increasing of N and P concentrations, which is similar to the previous study (Calvo-Fernández et al., 2018), because reduction of litter C/N and C/P ratio promoted the production of microbial extracellular phosphatases (Chen et al., 2018). However, AP activity decreased with increasing NP concentration, which may be closely related to changes in microbial habitat conditions caused by high concentrations of N and P additions in the short term (Jiang et al., 2018). With the extension of decomposition time, the soil enzyme activities of each treatment showed a trend of decreasing and then increasing, which was probably related to the change of climatic conditions in the study area. After the growing season, the rapidly decreases temperature and water content in the alpine region reduced the microbial activity, resulting in the decrease of the type and activity of litter decomposition enzymes.

5. Conclusion

Our result showed that N, P and NP addition could significantly affect the decomposition of litter matter, and P addition could alleviate the inhibitory effect of litter decomposition by high N concentration. N and P had an interactive effect on the decomposition of litter matter. The lignin remaining rate of litter was slightly enriched in the late stage of decomposition, which was most obvious in high N treatment, while the cellulose remaining rate decreased continuously with decomposition time. Litter C, N and P basically showed a continuous release pattern, with the strongest promoting effect in NP treatment. All additive treatments significantly increased soil invertase, cellulase, polyphenol oxidase, urease, and phosphatase activities, which were generally higher in the NP treatment than the other treatments. The rate of litter decomposition was significantly influenced by nutrient content as well as soil enzyme activity, where cellulose content and invertase activity may be a key factor controlling the rate of litter decomposition.

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