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Mixed-Species Plantation of *Pinus massoniana* Lamb. and *Quercus variabilis* Bl. and High Soil Nutrient Increase Litter Decomposition Rate

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Abstract: Changes in land use and forest planting have led to substantial changes in soil fertility and leaf litter input. The effects of mixed planting on the leaf litter decomposition rate in contrasting soil nutrient conditions are poorly understood. To elucidate the effects of litter composition and soil fertility on litter decomposition, we conducted a field litterbag-decomposition experiment with single (*Pinus massoniana* Lamb. or *Quercus variabilis* Bl.) and mixed (*P. massoniana* and *Q. variabilis*) litter treatments on soils of three nutrient levels (high, medium, and low). During the 3-year decomposition, at each decomposition stage and soil nutrient level, the mass-loss rate (MLR) was higher in mixed-litter than in the two single-litter treatments, with the exception of *Q. variabilis*, which recorded a higher MLR at 724 d in medium and high soil substrates. Between the two single-litter treatments, the MLR of *Q. variabilis* litter was higher than that of the *P. massoniana* litter; the MLR of the component litter of *P. massoniana* and *Q. variabilis* was higher than that of the corresponding two single-litter treatments. The *k* values over the 3-year-experiment period increased with the soil nutrient level for all litter treatments, as did microbial biomass carbon and nitrogen content. These findings suggest that mixed planting and high level of soil nutrient can accelerate litter decomposition.

Keywords: *k* value; microbial biomass; non-additive effect; soil enzyme; soil fertility

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1. Introduction

Decomposition is a critical process in terrestrial ecosystems, in that the release of materials from the decomposing litter can govern the availability of nutrients in the soil [1,2]. Litter decomposition rate, as one of the primary indexes for measuring decomposition characteristics, has historically been well studied in terms of *k* value (the decomposition constant, which stemmed from Olson 1963 [3]), and the current consensus argues that besides litter quality, interactions from climatic and soil conditions are the main regulating factors influencing it in situ [4,5]. The difference in coexisting species and complicated decomposition environment among plantations, however, frequently renders the decomposition characteristic unpredictable [6,7].

Mixed-species plantations obviously make sense for higher productivity and better ecological stability in ecosystems [8,9], a remarkable trait accounting for increased cases of mixture decomposition in situ for multiple co-existent species during afforestation and/or restoration compared with monoculture. Much evidence now points to differences in litter decomposition rates between litter in single- and mixed-species plantations [10], termed as the "non-additive effect", signifying that in no way can the decomposition rate of the

litter mixture be calculated directly from that of a single species in the mixture [11]. A set of theories plus these differences have been applied to interpret the mechanisms when changing the decomposition rate in multi-species ecosystems—these theories mainly deal with nutrient transfer, the effects of specific compounds, the interactions across trophic levels, and the improved micro-environmental conditions [12]. In addition, it has been indicated in previous studies that several potential methods may exist to influence the decomposition process in a given mixed plantation, such as changes in litter quality and alterations in the soil properties [13,14]. Theoretically, soil with higher available nutrients can maintain a higher decomposer activity in favor of the decomposition of the litter covering above (microbe could deploy enzymes extracellular to break down organic matter, which is sensitive to afforestation and nutrient addition) [15,16]. However, evidence shows that the response to fertilization with nutrients varies: litter decomposition may increase [17] or decrease, or show no difference [18]. The previously studied variations in litter species and climate biomes mean that the available results are inconclusive with regard to the control of the rates and dynamics and the non-additive performance involved in litter decomposition in plantation ecosystems with conditions of different soil nutrients.

Substantial changes in land use and land cover have taken place or are just underway in subtropical areas of China [19]. Deforestation and forest degradation there, together with agricultural development or soil erosion, have contributed to poor conditions of different soil nutrients. To restore the degraded land, a series of strategies are being employed correspondingly [20] so as to prompt a range of combinations of soil fertility conditions and leaf litter input. For example, in this area in the recent decade, afforestation or reforestation through planting monoculture forests of coniferous leaf species (e.g., *Pinus massoniana* Lamb.), broadleaf species (e.g., *Quercus variabilis* Bl.), or especially of mixtures with two or more species have been prevalent [21]. However, little information is available about how soil nutrient availability and litter quality (e.g., conifer and broadleaf) regulate decomposition, although it has been demonstrated that this process can be controlled by both soil substrate and litter quality [16,22].

From previous studies, we know that both the natural and planted forests of Masson pine and oak contribute to at least 7.7% and 10.4%, respectively, of the total forest area in China (widely distributed in subtropical China with monoculture or mixture), with relatively lower and higher rates of litter decay or nutrient cycling in situ, respectively [19,21,23]. For this study, we focused on the effects of litter mixture, soil nutrient substrate, and related micro-environmental factors of soil on the litter decomposition process based on an approximate three-year field experiment in a common subtropical forest site. We conducted a full factorial design for the litter decomposition experiment with different litter qualities (conifer, broadleaf, and mixture) in contrasting soil nutrient conditions (low, medium, and high substrates) in a mixed plantation of *Pinus massoniana* Lamb. and *Quercus variabilis* Bl. Our specific objectives were to determine the characteristics of the litter decomposition rate and its non-additive effect in response to soil substrates. We hypothesized that the litter decomposition rate and the non-additive mass loss rate would be higher for soil condition with a higher nutrient substrate.

2. Materials and Methods

2.1. Study Site Description

Our study site was located at the Forest Ecological Research Station in Zigui County (110°00′14″–111°18′41″ E, 30°38′14″–31°11′31″ N, elevation 40–2057 m a.s.l.), Hubei Province, China. The region experiences a subtropical monsoon climate which is dominated a mean annual temperature of 17–19 °C, and the monthly mean temperature ranges from −2.5 °C (January) to 44 °C (July and August); annual rainfall in the area is ~1000–1250 mm, mainly occurring from April to September. The soil in the study forests is Haplic luvisol soil. The area is dominated by both plantation of *Pinus massoniana* Lamb. and *Quercus variabilis* Bl., as well as a mixture of them, and natural secondary forests of *Cupressus funebris* Endl., *Cunninghamia lanceolata* (Lamb.) Hook., and *Pinus tabulaeformis* Carr. [23].

The experiment was conducted in a 38-year-old mixed-species plantation with *Pinus massoniana* and *Quercus variabilis*. Three 10 m × 10 m plots (at least 50 m apart) with similar canopy densities were set up in June 2018. The characteristics of understory vegetation were as follows: the shrub layer was dominated by *Camellia oleifera* Abel., *Loropetalum chinensis* (R. Br.) Oliv., and *Cotinus coggygria* Scop.; the herb layer is dominated by *Echinochloa crusgalli* (L.) Beauv. Ess. and *Veronicastrum villosulum* (Miq.) Yamazaki; lianas such as *Smilax china* L. and *Ficus martinii* H. Lév. et Vaniot are predominent in these plots.

2.2. Experimental Design

Newly senesced leaves of *P. massoniana* and *Q. variabilis* were directly collected from the floor of the research plantation in October–November 2018 (the green litter was sorted out). All leaf litter samples were taken back to the laboratory and air-dried (avoiding damaging the physical structure of the litter) for further use. In order to measure the effects of the soil nutrient substrates on litter decomposition, we collected soil of 0–5 cm depth from three locations where the initial soil chemistry was significantly different, and then the soil samples were sieved using a 2-mm mesh to remove leaves, plant roots, and stones, and they were homogenized into a pot (with diameter 25 cm and height 20 cm) with 1 kg according to three levels of soil nutrient substrates separately (Table 1).

Table 1. Initial soil chemistry of the three sites of *Pinus massoniana* Lamb. plantation.

Soil Nutrient Substrates	Sites	C (g·kg^{-1})	N (g·kg^{-1})	P (g·kg^{-1})	K (g·kg^{-1})
High	110°55′44.04″ E, 30°47′39.12″ N	567.23 ± 1.62 a	3.82 ± 0.01 a	0.38 ± 0.00 b	15.26 ± 0.06 a
Medium	110°58′42.96″ E, 30°49′44.40″ N	363.73 ± 2.05 b	2.18 ± 0.02 b	0.61 ± 0.00 a	5.94 ± 0.07 b
Low	110°56′30.84″ E, 30°50′13.92″ N	155.80 ± 3.20 c	0.63 ± 0.01 c	0.28 ± 0.00 c	5.38 ± 0.03 c

Different lowercase letters indicate a significant difference among soil substrates within the same variable ($p < 0.05$).

When the initial litterbags were prepared, each litter type with five samples was oven-dried in a condition of 65 °C for 48 h. The air-dried and the oven-dried mass ratio was used to convert the initial moisture content. Accordingly, air-dried litter samples of single and mixed species, totaling 10 g of dry weight for each litterbag (with an equal dry-weight ratio for mixtures), were placed in 15 cm × 15 cm litterbags with a nylon net of 1 mm mesh. In November 2018, the litterbags were distributed on the soil surface of the pot, and a total of 567 plastic pots with litterbags (3 soil nutrient substrates × 3 litter treatments × 3 plots × 3 replicates × 7 sampling events, with one litterbag per pot) were randomly placed in the established experimental plots (where the top 20 cm of the soil were removed). The litterbags were collected over the next three years corresponding to 85, 178, 268, 360, 541, 724, and 1090 days following the start of the experiment. In addition, on each sampling day, soil cores (0–5 cm deep) were collected directly below the decomposition litterbags from the pot, which were removed to assess the relationship between the soil traits and the litter decomposition.

2.3. Measurements of Soil Traits

Each processed soil sample (at least 300 g after mixed the three replicates in each plot) was divided into two portions, one was freshly sieved (2 mm mesh) and stored in a refrigerator at 4 °C for the analyses of the soil enzyme activity and microbial mass, and the other was oven-dried and finely ground to pass through a 0.25 mm sieve for the other chemical analyses.

Soil enzyme activities were measured using assay techniques modified from Guan (1986) [24]. In brief, the invertase activity was measured using colorimetry at 508 nm with 3,5-dinitrosalicylic acid. The cellulase activity was measured using colorimetry at 540 nm with 3,5-dinitrosalicylic acid. The soil urease activity assay was determined using the indophenol blue method. The polyphenol oxidase activity was measured using colorimetry

at 430 nm with absolute ether. The peroxidase activity was measured by incubating the soil with H_2O_2, ascorbic acid, and pyrocatecholand passivation enzyme phosphate.

The soil microbial biomass carbon was measured by fumigation with chloroform according to Vance et al. (1987) [25], and the other soil chemical properties were measured according to Lu (1999) [26]—the soil pH was determined by mixing the soil sample with deionized water at a 1:2.5 ratio (w/v); the soil organic matter content was measured by the wet digestion method with $K_2Cr_2O_7$; the soil total N content was analyzed using the Kjeldahl digestion procedure; the soil total P was measured by the NaOH fusion-molybdenum-antimony colorimetric method; and soil k was determined by the dissolved NaOH- flame photometric method.

2.4. Statistical Analysis

The mass-loss rates per phase were calculated as follows [11] with oven-dried litter mass at 65 °C to a constant weight (the remaining mass rate can be seen in Table S1):

$$MLR_t(\%) = \left(M_{(t-1)} - M_t\right)/M_{t0} \quad (1)$$

where $M_{(t-1)} - M_t$ represents the mass loss of corresponding litter on the current and previous sampling dates, and M_{t0} and M_t are the initial mass and remaining mass at time t, respectively.

The annual decay constant (k) was estimated using a single exponential decay model [3]:

$$y = ae^{-kt} \quad (2)$$

where y is the litter dry mass (remaining of initial), a is the fitting parameter, e is the natural constant, and t is the decomposition time (years).

The expected mass loss (E) of the litter mixtures was calculated as follows [10]:

$$E_t(\%) = (MLR_{At} + MLR_{Bt})/2 \quad (3)$$

where MLR_{At} and MLR_{Bt} are the measured mass loss rate of the two species in corresponding sample date.

After meeting assumptions of normality and homogeneity variance, analysis of variance (ANOVA) with Tukey's test was used to determine the differences of indexes investigated in the current experiment among the soil nutrient substrates (low, medium, and high substrates) or litter types (conifer, broadleaf, and mixture). An independent sample t-test was used to determine the difference in cases of every two samples, such as pairwise combinations, as shown in Figure 1. Exponential regression was used to fit the mass loss rate to the decomposition time. Univariate regression analysis was used with the litter mass loss rate or non-additive mass loss rate as a response variable and the initial soil traits were used as the predictor. The same method was adopted, except using the litter mass loss per phase as a response variable and the changing soil traits as the predictor. In addition, according to Gartner and Cardon (2004) [10], there is a non-additive effect when a significant difference occurs between the observed and expected mass-loss rate. We further defined the synergistic interaction (a positive nonadditive effect) when the observed mass loss minus the corresponding expected one was greater than zero.

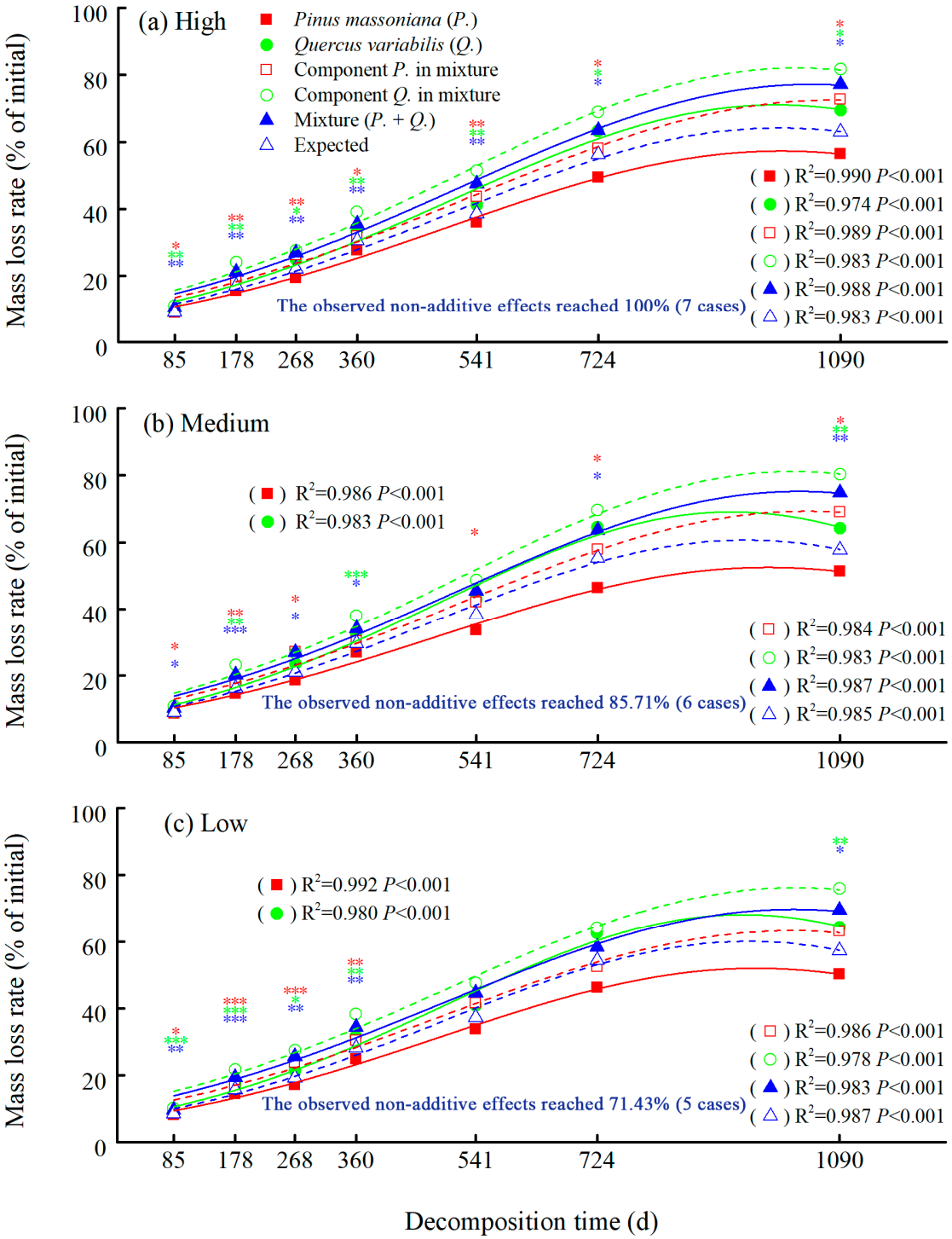


Figure 1. Dynamics of leaf litter decomposition in terms of mass loss rate in different soil nutrient substrates. The insets show results of independent samples *t*-test for pairwise combinations of the corresponding cases (they are between single *P.* and component *P.* in the mixture, between single *Q.* and component *Q.* in the mixture, as well as between the observed (mixture) and expected value, shown in the corresponding color) in the same sampling time. Statistical significance levels are ***, $p < 0.001$; **, $p < 0.01$; and *, $p < 0.05$. Inset percentages indicate the proportion of corresponding observed significant interactions (non-additive effect) in a total of seven cases for one soil condition.

3. Results

3.1. Leaf Litter Decomposition in Different Soil Substrates

The leaf litter mass loss increased as the decomposition proceeded, but differed among the litter type and the decomposition condition of the soil nutrient substrate (Figure 1). Over the 1090 days of incubation, 50.18% to 81.69% of the corresponding initial dry mass was lost among the single litter, the mixture, and the components litter in the mixture, with an increasing tendency for the loss of a specific litter type as the nutrients increased in the soil substrates. Compared with the litter type, the mass-loss rate (MLR) of *Q. variabilis* litter was larger than that of the *P. massoniana* litter, regardless of the soil substrates and decomposition phases. In addition, compared with the corresponding single litter type, both components of *P. massoniana* and *Q.s variabilis* in the mixtures showed higher MLR (regardless of soil nutrient substrate), which exhibited significant differences throughout the experiment time in the high soil nutrient substrate ($p < 0.05$ to 0.01) and at the most phases in the medium and low soil nutrient substrate ($p < 0.05$).

Compared with the MLR of the litter mixtures, the corresponding expected MLR was always lower (significantly lower during the first year regardless of soil nutrient substrate and in the second and third year for high soil nutrient substrate). There were 100%, 85.71%, and 71.43% observed incidence of non-additive MLR during the experiment period in the high, medium, and low conditions of the soil nutrient substrate, respectively.

Similarly, over the experiment period, the decomposition constant (k value) showed a significant difference among the leaf litter type and soil conditions (Figure 2). The litter k value of *P. massoniana* was always lower than that of *Q. variabilis*, regardless of the treatments of soil nutrient substrate and whether the comparison was of the single or among the components in the mixture ($p < 0.01$ to 0.001 for the three single cases; $p = 0.008$ to >0.05 for the three component cases, which was only significant in the treatment with a high soil nutrient substrate). In addition, the k value of the mixture was always larger than that of the two single litter types, regardless of the soil nutrient substrate (significance: $p < 0.05$, except for the two cases in the medium and low soil nutrient substrate for the comparison between the mixture and single litter of *Q. variabilis*). Compared with the excepted k value, the corresponding observed value in the three soil nutrient substrate treatments was significantly larger (significance: $p < 0.05$, except for the case in low soil nutrient substrate, which was equal to 0.05).

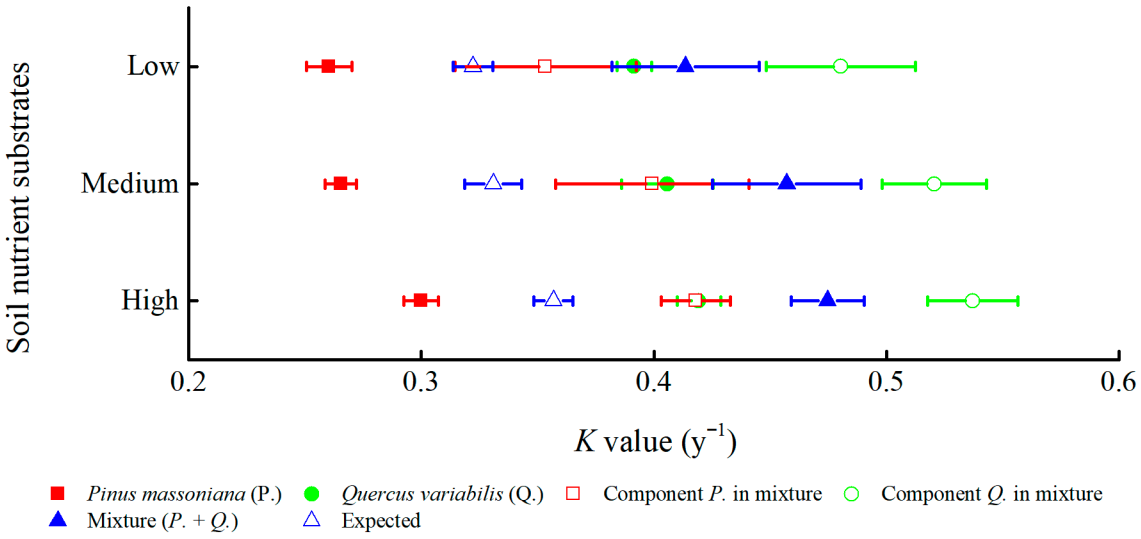


Figure 2. Effect of soil nutrient substrate on leaf litter decomposition (in terms of decomposition constant: k value). Values are the mean ± SE ($n = 3$). The results of two-way ANOVA suggest significant effects from the soil nutrient substrate ($F_{(2,18)} = 4.142$, $p = 0.033$) and leaf litter type ($F_{(2,18)} = 73.533$, $p < 0.001$) on the decomposition constant.

3.2. Dynamics of Soil Properties and Their Effects on Leaf Litter Decomposition

The microbial mass carbon and nitrogen showed a significant difference ($p < 0.001$) among soil conditions, with a significantly higher value in the high soil nutrient substrate (Figure 3). Among the litter types, only the two cases observed significant differences ($p < 0.05$ to 0.01) at the high soil nutrient substrate (when the decomposition proceeded at 724 and 1090 days for the MBC and MBN, respectively). In addition, as the decomposition proceeded, the changes in the other investigated soil traits did not show a significant difference ($p > 0.05$) among the litter types, but significantly varied in the treatments of the soil condition ($p < 0.05$ to 0.001), regardless of the decomposition phase (Figures S1 and S2).

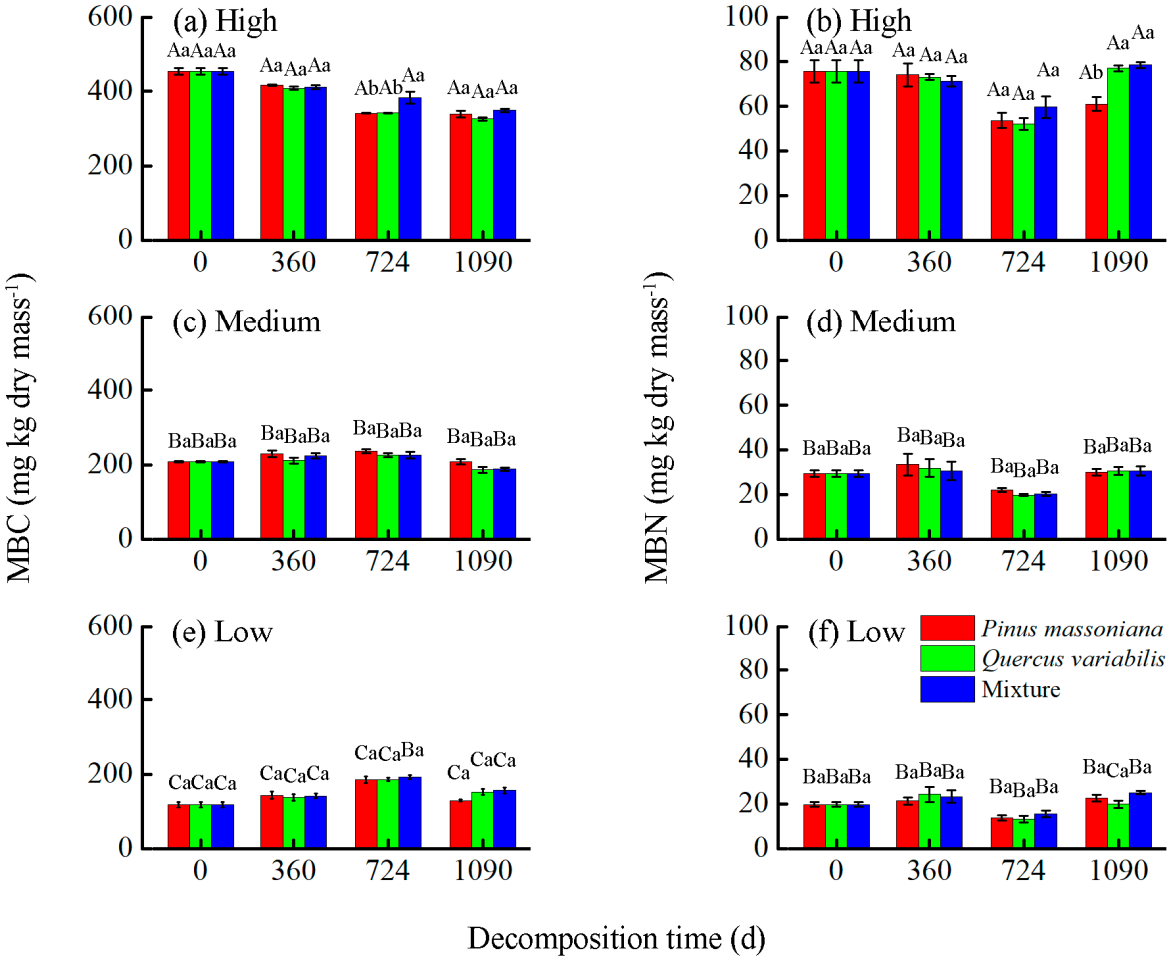


Figure 3. Dynamic of soil microbial biomass for carbon (**a**,**c**,**e**) and microbial biomass nitrogen (**b**,**d**,**f**) among the litter types in different soil nutrient substrates during decomposition. Values are the mean ± SE ($n = 3$). Different capital letters indicate a significant difference among soil substrates for the same litter type at the same decomposition time; different lowercase letters indicate a significant difference among litter types for the same soil substrate at the same decomposition time ($p < 0.05$).

The initial soil traits investigated in this study had a significant influence on the MLR of two single litter types during the first year and the entire three years, with the exception of initial soil phosphorus (Table 2). In addition, changing the soil traits had significant effects on the litter MLR in the phases (regardless of litter type for most phases), especially during the phases of 0–85 d, 86–178 d, and the third year of the experiment time (Table 3).

Table 2. Regression analyses of initial soil traits and litter mass loss rate or non-additive mass loss rate after one year (A), two years (B), and three years (C).

	Index	Carbon	Nitrogen	Phosphorus	K	PH	Polyphenol Oxidase	Peroxidase	Sucrase	Cellulase	Urease	MBC	MBN
(A)	Mass loss of *Pinus massoniana* (P.)	(+)34.653 ** (0.832)	(+)32.536 ** (0.823)	2.308 (0.225)	(+)7.223 * (0.508)	(−)24.048 ** (0.775)	(+)17.456 ** (0.714)	(−)38.334 *** (0.846)	(+)14.004 ** (0.667)	(+)19.918 ** (0.740)	2.635 (0.273)	(+)14.925 ** (0.681)	(+)10.595 * (0.602)
	Mass loss of *Quercus variabilis* (Q.)	(+)11.887 * (0.629)	(+)11.850 * (0.629)	0.465 (0.062)	(+)6.671 * (0.488)	(−)11.328 * (0.618)	(+)10.327 * (0.596)	(−)8.036 * (0.534)	(+)9.471 * (0.575)	(+)10.782 * (0.606)	3.401 (0.327)	(+)9.728 * (0.582)	(+)8.295 * (0.542)
	Mass loss of component P. in mixture	0.501 (0.067)	0.520 (0.069)	0.093 (0.013)	0.811 (0.104)	0.599 (0.079)	0.672 (0.088)	0.174 (0.024)	0.716 (0.093)	0.643 (0.084)	0.834 (0.106)	0.704 (0.091)	0.763 (0.098)
	Mass loss of component Q. in mixture	0.915 (0.116)	0.962 (0.121)	0.367 (0.050)	1.870 (0.211)	1.173 (0.143)	1.382 (0.165)	0.220 (0.030)	1.519 (0.178)	1.297 (0.156)	2.114 (0.232)	1.480 (0.175)	1.680 (0.194)
	Mass loss of mixture	0.702 (0.091)	0.732 (0.095)	0.181 (0.025)	1.247 (0.151)	0.864 (0.110)	0.988 (0.124)	0.212 (0.029)	1.065 (0.132)	0.938 (0.118)	1.330 (0.160)	1.044 (0.130)	1.152 (0.141)
	Non-additive mass loss	1.573 (0.183)	1.486 (0.175)	2.908 (0.293)	0.236 (0.033)	1.130 (0.139)	0.822 (0.105)	3.300 (0.320)	0.641 (0.084)	0.942 (0.119)	0.002 (0.000)	0.691 (0.090)	0.444 (0.060)
(B)	Mass loss of *Pinus massoniana*	2.621 (0.272)	2.709 (0.279)	0.095 (0.013)	3.837 (0.354)	3.078 (0.305)	3.396 (0.327)	1.064 (0.008)	3.572 (0.338)	3.274 (0.319)	3.486 (0.332)	3.525 (0.335)	3.735 (0.348)
	Mass loss of *Quercus variabilis*	0.129 (0.018)	0.110 (0.016)	1.917 (0.215)	0.051 (0.007)	0.045 (0.006)	0.009 (0.001)	0.699 (0.091)	0.000 (0.000)	0.020 (0.003)	0.307 (0.042)	0.001 (0.000)	0.008 (0.001)
	Mass loss of component P. in mixture	1.793 (0.204)	1.738 (0.199)	1.080 (0.134)	0.641 (0.084)	1.492 (0.176)	1.244 (0.151)	2.463 (0.260)	1.081 (0.134)	1.345 (0.161)	0.197 (0.027)	1.128 (0.139)	0.885 (0.112)
	Mass loss of component Q. in mixture	5.615 (0.445)	5.298 (0.431)	5.176 (0.425)	1.138 (0.140)	4.044 (0.366)	3.014 (0.301)	(−)12.521 ** (0.641)	2.429 (0.258)	3.409 (0.328)	0.194 (0.027)	2.589 (0.270)	1.806 (0.205)
	Mass loss of mixture	3.153 (0.311)	3.027 (0.302)	2.265 (0.244)	0.894 (0.113)	2.484 (0.262)	1.981 (0.221)	5.062 (0.420)	1.668 (0.192)	2.181 (0.238)	0.216 (0.030)	1.756 (0.201)	1.311 (0.158)
	Non-additive mass loss	2.749 (0.282)	2.583 (0.270)	5.217 (0.427)	0.400 (0.054)	1.926 (0.216)	1.381 (0.165)	(−)6.506 * (0.482)	1.071 (0.133)	1.591 (0.185)	0.008 (0.001)	1.156 (0.142)	0.743 (0.096)
(C)	Mass loss of *Pinus massoniana*	(+)18.963 ** (0.730)	(+)20.163 ** (0.742)	0.015 (0.002)	(+)31.690 ** (0.819)	(−)25.778 ** (0.786)	(+)31.036 ** (0.816)	5.262 (0.429)	(+)33.539 ** (0.827)	(+)29.030 ** (0.806)	(+)16.978 ** (0.708)	(+)32.948 ** (0.825)	(+)34.541 ** (0.831)
	Mass loss of *Quercus variabilis*	(+)8.028 * (0.534)	(+)8.507 * (0.549)	0.297 (0.041)	(+)18.641 ** (0.727)	(−)10.807 ** (0.607)	(+)11.311 ** (0.655)	2.233 (0.242)	(+)15.005 ** (0.682)	(+)12.275 * (0.637)	(+)16.263 ** (0.699)	(+)14.524 ** (0.675)	(+)16.924 ** (0.707)
	Mass loss of component P. in mixture	2.855 (0.290)	2.828 (0.288)	0.403 (0.054)	1.711 (0.196)	2.668 (0.276)	2.451 (0.259)	2.634 (0.273)	2.280 (0.246)	2.545 (0.267)	0.929 (0.117)	2.331 (0.250)	2.046 (0.226)
	Mass loss of component Q. in mixture	3.292 (0.320)	3.226 (0.315)	0.861 (0.110)	1.536 (0.180)	2.898 (0.293)	2.534 (0.266)	3.653 (0.343)	2.279 (0.246)	2.686 (0.277)	0.683 (0.089)	2.353 (0.252)	1.957 (0.218)
	Mass loss of mixture	3.730 (0.348)	3.677 (0.344)	0.637 (0.083)	1.978 (0.220)	3.390 (0.326)	3.040 (0.303)	3.676 (0.344)	2.780 (0.284)	3.189 (0.313)	0.984 (0.123)	2.856 (0.290)	2.440 (0.258)
□	Non-additive mass loss	0.372 (0.050)	0.335 (0.046)	2.707 (0.279)	0.004 (0.001)	0.195 (0.027)	0.093 (0.013)	1.330 (0.160)	0.045 (0.006)	0.130 (0.018)	0.198 (0.027)	0.057 (0.008)	0.009 (0.001)

Values are *F*-value and determination coefficient (R^2, in parenthesis) for regression analysis with mass loss or non-additive mass loss as response variables and initial soil traits as predictor variables. (+), positive relationship; (−), negative relationship; significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; $n = 9$.

Table 3. Regression analyses of changing soil traits and the litter mass loss rate (loss of initial per phase).

Decay Phases	Index	Carbon	Nitrogen	Phosphorus	K	PH	Polyphenol Oxidase	Peroxidase	Sucrase	Cellulase	Urease	MBC	MBN
0 d-	Mass loss of *Pinus massoniana*	(+)19.618 ** (0.737)	(+)18.594 ** (0.726)	2.464 (0.260)	4.860 (0.410)	(−)14.403 ** (0.673)	(+)10.931 * (0.610)	(−)27.306 ** (0.796)	(+)8.994 * (0.562)	(+)12.258 * (0.637)	1.746 (0.200)	(+)9.521 * (0.576)	(+)6.977 * (0.499)
85 d	Mass loss of *Quercus variabilis*	(+)14.692 ** (0.677)	(+)13.743 ** (0.663)	4.409 (0.386)	3.029 (0.302)	(−)10.168 * (0.592)	(+)7.462 * (0.516)	(−)34.178 ** (0.830)	(+)6.015 * (0.462)	(+)8.476 * (0.548)	0.875 (0.111)	(+)6.405 * (0.478)	4.544 (0.394)
	Mass loss of mixture	(+)7.116 * (0.504)	(+)6.935 * (0.498)	1.328 (0.159)	2.887 (0.292)	(−)6.064 * (0.464)	5.146 (0.424)	(−)7.908 * (0.530)	4.531 (0.393)	5.522 (0.441)	1.224 (0.149)	4.707 (0.402)	3.794 (0.351)
86 d-	Mass loss of *Pinus massoniana*	2.705 (0.279)	4.419 (0.387)	1.254 (0.152)	(−)13.586 ** (0.660)	(−)6.993 * (0.500)	(+)14.843 ** (0.680)	2.508 (0.264)	(+)11.381 * (0.619)	3.485 (0.332)	(+)14.432 ** (0.673)	—	—
178 d	Mass loss of *Quercus variabilis*	(+)24.013 ** (0.774)	(+)32.059 ** (0.821)	0.063 (0.009)	(+)31.229 ** (0.817)	(−)38.274 *** (0.845)	(+)23.535 ** (0.771)	(−)11.902 * (0.630)	(+)35.667 ** (0.836)	(+)29.363 ** (0.807)	3.678 (0.344)	—	—
	Mass loss of mixture	(+)8.610 * (0.552)	(+)10.699 * (0.604)	0.005 (0.001)	(+)12.532 ** (0.642)	(−)12.528 ** (0.642)	(+)11.798 * (0.628)	3.586 (0.339)	(+)13.035 ** (0.651)	(+)19.722 * (0.581)	2.764 (0.283)	—	—
179 d-	Mass loss of *Pinus massoniana*	2.492 (0.263)	1.858 (0.210)	2.049 (0.226)	0.366 (0.050)	1.048 (0.130)	0.414 (0.056)	1.749 (0.200)	0.620 (0.081)	1.404 (0.167)	0.064 (0.009)	—	—
268 d	Mass loss of *Quercus variabilis*	3.328 (0.322)	3.312 (0.321)	0.615 (0.081)	1.775 (0.202)	2.793 (0.285)	1.722 (0.197)	3.264 (0.318)	2.197 (0.239)	3.101 (0.307)	0.313 (0.043)	—	—
	Mass loss of mixture	0.076 (0.011)	0.199 (0.028)	0.405 (0.055)	0.764 (0.098)	0.373 (0.051)	0.771 (0.099)	0.132 (0.019)	0.636 (0.083)	0.269 (0.037)	1.110 (0.137)	—	—
269 d-	Mass loss of *Pinus massoniana*	0.678 (0.088)	0.548 (0.073)	0.461 (0.062)	0.175 (0.024)	0.115 (0.016)	0.423 (0.057)	0.462 (0.062)	0.280 (0.038)	0.486 (0.065)	0.015 (0.002)	0.389 (0.053)	0.315 (0.043)
360 d	Mass loss of *Quercus variabilis*	1.484 (0.175)	1.404 (0.167)	0.286 (0.039)	0.806 (0.103)	0.763 (0.098)	0.981 (0.123)	1.293 (0.156)	1.005 (0.126)	1.356 (0.162)	0.060 (0.008)	1.148 (0.141)	0.978 (0.123)
	Mass loss of mixture	0.370 (0.050)	0.039 (0.005)	(−)9.015 * (0.563)	0.606 (0.080)	0.998 (0.125)	0.827 (0.106)	0.021 (0.003)	0.256 (0.035)	0.007 (0.001)	5.180 (0.425)	0.046 (0.007)	0.296 (0.041)
361 d-	Mass loss of *Pinus massoniana*	0.028 (0.004)	0.290 (0.040)	(−)12.514 * (0.641)	0.215 (0.030)	0.744 (0.096)	1.013 (0.126)	0.173 (0.024)	0.000 (0.000)	0.073 (0.010)	3.279 (0.319)	—	—
541 d	Mass loss of *Quercus variabilis*	0.932 (0.117)	0.600 (0.079)	0.578 (0.076)	1.466 (0.173)	0.168 (0.023)	1.772 (0.202)	0.616 (0.081)	1.034 (0.129)	0.762 (0.098)	1.865 (0.210)	—	—
	Mass loss of mixture	0.968 (0.121)	1.040 (0.129)	0.116 (0.016)	0.705 (0.091)	1.058 (0.131)	0.442 (0.059)	0.997 (0.125)	0.924 (0.117)	0.993 (0.124)	0.113 (0.016)	—	—
542 d-	Mass loss of *Pinus massoniana*	0.519 (0.069)	0.516 (0.069)	0.029 (0.004)	0.575 (0.076)	0.591 (0.078)	0.588 (0.078)	0.537 (0.071)	0.590 (0.078)	0.568 (0.075)	0.556 (0.074)	—	—
724 d	Mass loss of *Quercus variabilis*	0.037 (0.005)	0.024 (0.003)	0.047 (0.007)	0.138 (0.019)	0.099 (0.014)	0.111 (0.016)	0.035 (0.005)	0.099 (0.014)	0.052 (0.007)	0.079 (0.011)	—	—
	Mass loss of mixture	1.468 (0.173)	2.018 (0.224)	(+)22.892 ** (0.766)	0.044 (0.006)	0.039 (0.006)	0.005 (0.001)	2.196 (0.239)	0.337 (0.046)	0.804 (0.103)	0.402 (0.054)	—	—
725 d-	Mass loss of *Pinus massoniana*	(+)5.897 * (0.457)	(+)6.203 * (0.470)	0.256 (0.035)	5.487 (0.439)	(−)6.126 * (0.467)	(+)5.901 * (0.457)	(−)6.192 * (0.469)	(+)6.104 * (0.466)	(+)6.381 * (0.477)	(+)6.494 * (0.481)	(+)6.488 * (0.481)	(+)6.196 * (0.470)
1090 d	Mass loss of *Quercus variabilis*	4.863 (0.410)	4.117 (0.370)	0.647 (0.085)	(+)35.176 ** (0.834)	(−)13.234 ** (0.654)	(+)6.791 * (0.492)	(−)13.542 ** (0.659)	(+)24.778 ** (0.780)	(+)17.044 ** (0.709)	(+)10.939 * (0.610)	(+)14.791 ** (0.679)	(+)20.968 ** (0.750)
□	Mass loss of mixture	1.115 (0.137)	1.028 (0.128)	0.008 (0.001)	1.693 (0.195)	1.474 (0.174)	1.270 (0.154)	1.278 (0.154)	1.612 (0.187)	1.447 (0.171)	1.506 (0.177)	1.603 (0.186)	1.651 (0.191)

Values are *F*-value and determination coefficient (R^2, in parenthesis) for regression analysis with mass loss as response variables and changes in soil traits as predictor variables. (+), positive relationship; (−), negative relationship; significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; $n = 9$.

4. Discussion

With the expectation that the litter mass loss rate (MLR) of *Q. variabilis* was larger than that of *P. massoniana* during this experiment, our results are consistent with the current consensus that litter of a higher quality (initial litter chemistry can be found in Figure S2) can decompose faster than that litter of a lower quality [27,28]. In addition, our data confirmed the first hypothesis that leaf litter decomposition would have a larger rate on soil condition with a higher nutrient substrate than that of soil with a relatively lower nutrient substrate. Absolutely, decomposers in the high-nutrient substrate can obtain the nutrients they need not only from the litter, but also from the soil where the nutrients are relatively more available, in order to support their activities [13]. Our findings show that the MBC and MBN were significantly higher in the high soil substrate than in the condition of the others (Figure 3). Accordingly, the integration of abundant labile compounds and the higher microbial activities in the higher soil condition tended to make the litter have a relatively high decomposition rate (with larger *k* values).

According to the conclusions of non-additive effects reviewed by Gartner and Cardon (2004) [10], 67% of previous studies argued that there was a non-additive mass loss during litter decomposition. Our results coincide with the notion suggesting mixed planting with *P. massoniana* and *Q. variabilis* can accelerate the decomposition rate in situ as a result of our findings that the *k* value of litter mixtures was larger than that of the single-litter treatment, regardless of the soil nutrient conditions (Figure 2). Besides this, interestingly, our findings show that the investigated two litter types exhibited a relatively higher MLR and *k* value in the litter mixtures, compared with the corresponding single litter treatments in every specified soil condition. The underlying mechanism can be addressed, in that in a mixed-species litterbag, the scarce materials the decomposers need can transfer from nutrient-rich litter to nutrient-poor litter [29]. In such a "litter-mix ecosystems" (a microenvironment with nutrients relatively more available compared with "single-litter ecosystems"), the decomposers can obtain nutrients without a limitation from each specified species, which in turn benefits the decomposition of each of the other components [30]. The present results also suggest that with the soil contained an initial microbial community that existed in *P. massoniana* forest (relates to the decomposition of species with low litter quality [31]), the decomposition controlled by the litter quality could be facilitated [32], further showing it is suitable to afforest with the two species resulting from the synergistic effect.

However, our hypothesis that the non-additive mass loss rate would increase along with the increase in the nutrient level of soil substrates was not well supported by our data. The values (the mixture minus the corresponding result expected in Figure 1) were not always in the following order, the high substrate > in the medium substrate > in the low substrate, as the decomposition proceeded. However, importantly, we observed a decreased incidence of the non-additive effect from the high soil substrate to the low soil substrate (means for a specified phase, the non-additive effect can be found in the treatment of high soil nutrient substrate, but might not occur in the treatments of the medium or low soil nutrient substrate). From previous studies, we know that component species in a mixture with superior litter quality can promote the decomposition of "litter-mix ecosystems", while the mixture of a low-quality litter usually leads to antagonistic interactions (which are not beneficial to decomposition) [33,34]. Considering the synergistic effects we found among the two species in a mixture, a reasonable explanation for the difference in findings of non-additive mass loss rates among the soil substrates was that the observed mass loss rate was a net value resulting from multiple processes, the synergistic or antagonistic interactions, and the magnitude of these multiple processes was regulated by the decomposition time (in terms of changing soil traits, Table 3 indicates the changes in the microenvironment of decomposition) [35,36]. Compared with our treatments on soil nutrient conditions, the incidence of these interactions in the high soil substrate might have been more frequent and, with the relatively abundant soil nutrients available for decomposers, finally showed a significant net synergistic effect during decomposition (Figure 1) [32].

According to Delgado-Baquerizo et al. (2015) [17], the soil characteristics stemming from the semi-arid roadside grasslands of central Spain determined the soil carbon and nitrogen availability during decomposition, regardless of the litter quality. This indicated that the soil condition had a greater effect than litter quality on-site for determining the litter decomposition. Our findings show, however, that although the soil traits (no matter the initial traits or the changing dynamics as the decomposition proceeded) had a significant influence for most of the specified times on the litter mass loss rate in terms of the accumulated (Table 2) and the phased results (Table 3), no significant relationship was shown to exist between the soil traits and litter mass loss during the second year of the experiment. The following underlying mechanisms explain the observed pattern: (1) litter decomposition is a continuous process, the subsequent characteristic of mass loss is regulated by changes in litter quality resulting from the last decomposition phase [22]; (2) on the other hand, the response of soil characteristics to litter nutrient release, in turn, might lead to a profound influence on litter mass loss [37]. Therefore, considering the soil nutrient substrates evaluated in our experiment, the present inconsistencies might be attributed to the soil, in that it can maintain luxuriant nutrients to support microbial activities, although in a low nutrient condition (at least 1 kg soil with 35 to 133 times carbon compared with that in 10 g of dry weight litter for a specified pot, not shown). This is why there is no significant difference in soil traits among litter types, but there is among the treatments of the soil nutrient substrate (Figures S1 and S2). The several cases of the significant relationship between the non-additive mass loss rate and the initial soil traits, shown in Table 2, may be related to the "dilution effect" theory [38].

5. Conclusions

Our experiment on a mixed plantation of *P. massoniana* and *Q. variabilis.* showed that both the litter quality and soil nutrient level can significantly influence the litter mass-loss rate. The higher-quality litter had higher decomposition rates. As the nutrient level of the soil increased, the litter mass-loss rate increased. In addition, at a given soil nutrient level, mixed-species treatment not only had higher mass-loss rates than the two single-litter types, but also had higher MLR of components (in mixtures) than that of the corresponding single-litter type. We also found that the incidence of non-additive MLR would increase as the level of nutrients in the soil increased. Overall, these findings suggested mixed planting with *P. massoniana* and *Q. variabilis* could accelerate mass loss for each other, with higher soil nutrient substrates increasing decomposition. Further work investigating variations in litter chemistry traits and their underlying mechanisms responding to soil substrates would be helpful to improve the understanding of afforestation or reforestation with various soil conditions under the scenario of land-use change.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14040708/s1. Figure S1: Dynamics of investigated soil enzymes adjacent litterbags during decomposition (in terms of incubation day); Figure S2: Dynamics of soil chemical traits adjacent litterbags during decomposition (in terms of incubation day); Table S1: Remaining mass rate (% of initial) of litter in different soil conditions (nutrient substrate) during decomposition (in terms of incubation day).

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