Functional diversity of decomposers modulates litter decomposition affected by plant invasion along a climate gradient

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Abstract
1. Litter decomposition is fundamental to carbon (C) and nutrient cycling in ecosystems, which could be altered by plant invasion. The impacts of plant invasion on litter decomposition are generally predicted by trait difference between leaf litters of invasive and non-invasive species. However, plant invasion not only changes litter composition, but might also increase the activity or change the functional diversity of decomposers to alter litter decomposition, which is barely studied, and the effect could be different under varied climate conditions.
2. We studied decomposition of litters from non-invasive and invasive native plants, as affected by litter treatments (in a mixture or alone) and decomposer organisms of different functional groups (by controlling the mesh size of litterbags), in sites with or without an invasive woody grass, Moso bamboo Phyllostachys edulis, at seven locations across a climate gradient.
3. We show that greater decomposer functional diversity, particularly the presence of macrofauna, accelerated the cycling of litter C and nitrogen (N), increased the climatic sensitivities of decomposition rates, but decreased the N use efficiency of decomposers (represented by litter C to N loss ratio). Litter decomposed in mixtures decomposed faster (by 9.5%) and had more N loss (by 28.9%) than that of the monoculture, regardless of the functional diversity of decomposers. In contrast, the invasion of Moso bamboo slowed decomposition and decreased N use efficiency; this negative effect could be reversed when macrofauna were excluded from the decomposition process, which challenges the nutrient facilitation hypothesis. Bamboo invasion depressed the climatic sensitivity of decomposer functional groups when macrofauna were present but not when macrofauna were excluded.
1 | INTRODUCTION

Exotic invasive species are a global concern because of their significant impact on ecosystem processes and functions (Ehrenfeld, 2010; Vilà et al., 2011). The effect of plant invasion on litter decomposition is a concern because it impacts ecosystem functioning (Schlesinger & Bernhardt, 2013; Wardle, 2002). By affecting the cycling of carbon (C) and availability of nutrients, for example, nitrogen (N), litter decomposition could influence plant growth and community structure (Hobbie, 1992, 2015; Wardle, 2002). However, past studies have focused more on differences in traits between invasive and non-invasive plant species to predict ecosystem functioning (e.g. litter decomposition) altered by invasion (Jo et al., 2016; Martin et al., 2017; Prescott & Zukswert, 2016; Van Kleunen et al., 2010), and a functional trait-based framework has also been proposed (Helsen et al., 2018), while multiple factors, including microclimate, litter composition (variation in litter quality) and soil biota that control litter decomposition (Bradford et al., 2016) could be altered by plant invasion. How these plant invasion induced hierarchical changes affect C and nutrient cycling (Bardgett et al., 2013), and respond to potential changes in climate, the main driver of litter decomposition (Bradford et al., 2016), are less understood. Therefore, there is a need for systematic studies that can simultaneously consider the effects of litter quality and soil biota in ecosystems where plant invasion is occurring, under different climate conditions.

Leaf litter traits of exotic plant species (Ehrenfeld, 2010) are known to drive litter decomposition (Jo et al., 2016; Van Kleunen et al., 2010). The generalization is that the litter of exotic plants decomposes faster than the litter of co-occurring native plants under controlled conditions (Ehrenfeld, 2003; Liao et al., 2008), which increases nutrient availability and could support faster growth of the exotic plants (Belnap et al., 2005; Holdredge & Silliman, 2010; Jo et al., 2017). However, litters from multiple plant species are decomposed in a mixture in forests, and litter-mixing may have synergistic or antagonistic effects on the decay rate of mixed litter as compared to litter of a single species (Lecerf et al., 2011; Wardle et al., 2003), but this litter-mixing effect is little studied in ecosystems experiencing plant invasion (Hickman et al., 2013). Furthermore, decomposition and nutrient cycling could be affected by alteration to the physical habitat (Ashton et al., 2005), another mechanism that is employed by exotic invaders to outcompete the native flora (Crooks, 2002). For example, salt cedar (Tamarix spp.), a deep-rooted plant, can lower the water table by increasing transpiration rates to inhibit the growth of native plants and animals (Randall, 1996); and exotic willows can decrease macrofaunal density and species richness by reducing habitat complexity (Read & Barmuta, 1999). Therefore, ignoring the effects of litter-mixing and habitat-alteration may lead to erroneous conclusions on how invaders modulate decomposition. In addition, how climate factors may influence the litter-mixing and habitat-alteration effects of invaders and thereby change litter decomposition is unknown, given that climatic conditions (e.g. temperature and moisture) exert a strong influence on litter decomposition (Bradford et al., 2016).

Litter decomposition is mediated by decomposer organisms that inhabit the soil and litter layer, including micro-organisms and invertebrate consumers of varying body sizes that represent functional diversity of decomposers. Decomposer organisms exert significant effect on litter decomposition (Gessner et al., 2010; Handa et al., 2014; Hättenschwiler & Gasser, 2005) and provide key ecosystem functions (Bardgett & van der Putten, 2014). Decomposer organisms are responsive to environmental changes at the microsite scale, and will likely be affected by the alteration of habitat by invasive plants. For example, soil biota of different functional groups, macro-detritivores (Mayer et al., 2005), arthropods (Simao et al., 2010), nematodes (Belnap et al., 2005), and fungi (Kivlin & Hawkes, 2011) were affected by invasive plants because of the changed palatability of their litter to detritivores (Bastow et al., 2008), or the microbial community may become adapted to decompose novel compounds in the litter of invasive plants (Lankau et al., 2009). Invasive plants can increase bacterial biomass by 16%, detritivore abundance by 119%, and microbivore abundance by 89% through litter input (Zhang et al., 2019), but Vilà et al. (2011) synthesized that invasive plants generally reduce the abundance of animal species, while have varying effects on their diversity. Though effects of plant invasion on litter decomposition...
have been widely studied (Ashton et al., 2005; Mayer et al., 2005; Prescott & Zukswert, 2016), the soil decomposer community was mostly considered as a whole (Figure 1). Yet, how soil biota from different functional groups alter litter decomposition under the impact of plant invasion, remains to be studied. It has been demonstrated that the effect of soil fauna on litter decomposition is differently modulated by climate and litter quality (García-Palacios et al., 2013; Hättenschwiler & Gasser, 2005). Furthermore, emerging evidence shows that plant invasion alters trophic interactions at higher trophic levels (e.g. carnivores and herbivores, Devore & Maerz, 2016; Simao et al., 2010) and induces cascading effects for ecosystem function. How trophic cascade in the soil food web interacts with subsequent changes in litter quality after plant invasion to change decomposition processes needs to be clarified, under different climate conditions.

Bamboo invasion is occurring world-wide, both in its native habitats, for example, Brazil (Lima et al., 2012) and China (Mertens et al., 2008), as well as in its new habitats, for example, Japan (Suzuki & Nakagoshi, 2008). Due to its commercial value, Moso bamboo *Phyllostachys edulis*, a native species in China, was widely planted and accounted for more than 70% of the nation's bamboo forest area (i.e. 6.41 million ha, data from the ninth national inventory of forest resources of China). But lack of management and its fast clonal growth lead to the spreading of Moso bamboo to other forests (Xu et al., 2020), affecting soil microbial communities and diversity (Xu et al., 2015), and soil C and N cycling (Li et al., 2017). Species that expand across their native range and outcompete other natives in response to human-mediated disturbances (Simberloff, 2011), such as fire suppression (e.g. *Acer rubrum* L in deciduous forests), and livestock grazing (e.g. *Juniperus occidentalis* in sagebrush; Carey et al., 2012), are named ‘native invaders’. Although they can cause similar harmful ecological and economic impacts as exotics (Carey et al., 2012), we have limited knowledge of how native invaders alter ecosystem processes, and if the mechanisms explaining the expansion of exotic invasive species apply to native invaders as well. The objective of this study was to unveil how a native invader, Moso bamboo, affecting litter decomposition, with the mediation of decomposer organisms through litter-mixing and habitat-alteration, under different climatic conditions. We hypothesize that (a) both litter-mixing and habitat-alteration stimulate litter decomposition; (b) The presence of large-bodied soil fauna (macrofauna) exert the largest impacts on decomposition and responds most sensitively to climate; (c) Functional diversity of decomposers (variation in body size) interactively affect litter decomposition with litter-mixing and habitat-alteration, but are climate dependent.

### 2 | MATERIALS AND METHODS

#### 2.1 | Experimental sites and design

Our study was accomplished by deploying more than 3,000 litterbags at seven locations along a climatic gradient with mean
annual temperature (MAT) ranging from 12 to 20°C and mean annual precipitation (MAP) ranging between 1,043 and 2,144 mm (Table S1; Figure S1). The seven research sites were selected from the nation-wide bamboo distribution area where bamboo invasion has been reported. The sites span the warm temperate region in the north to the subtropical region in the south (Table S1; Figure S1). The sites include monsoon climate in the transition from northern subtropical zone to warm temperate zone (e.g. Henan), subtropical monsoon climate (e.g. Anhui, Zhejiang, Sichuan, Fujian and Jiangxi) and south subtropical monsoon humid climate (e.g. Guangdong). The zonal forests were oak-dominated deciduous broad-leaved forest in Henan, and subtropical monsoon evergreen broad-leaved forest in the other study areas. At each location, four replicated transects were set up to represent a bamboo invasion gradient, that is, pure bamboo forest, native forest community invaded by bamboo, and non-invaded native forest community.

The field experiment in the seven different locations followed an identical protocol. The litterbags were reciprocal transplanted in different forest types (incubation habitat), that is, where the invasive Moso bamboo was present or absent (effect of habitat-alteration), and litterbags contained different litter types (alone or mixed litters from the bamboo and native trees at the location, effect of litter-mixing), and restricted access to functional groups of decomposers by controlling the mesh size (effect of functional diversity of decomposers). Across all seven locations, and in three forest types representing a bamboo invasion gradient, the experiment used a randomized block design, with leaf litters enclosed in nylon mesh screens and placed in the field in four blocks (n = 7 locations × 3 forest types × 4 blocks × 3 litter combinations × 3 mesh sizes × 4 harvestings = 3,024 litterbags). Three litter combinations were a single species location-specific (i.e. collected from the study site) bamboo, a single species location-specific native-dominant tree species (hereafter called native tree species) and the mixture of the two location-specific litter types. Three mesh sizes (50 µm, 1 and 5 mm) were used to distinguish three increasingly completeness of the decomposer communities (small-sized, medium-sized and large-sized decomposer organisms) that colonized or consumed the decomposing litter. Small decomposers included micro-organisms and small-sized fauna that can pass through 50-µm mesh screens (excluded the mesofauna and macrofauna). The medium-sized decomposer communities contained all organisms (including invertebrates) that can pass through 1-mm mesh screens (macrofauna were excluded), whereas the large-sized decomposer communities included all decomposers that can pass through 5-mm mesh screens.

2.2 | Leaf litter collection and field incubations

A total of 14 litter types were collected from the seven locations. The selected species were bamboo and paired native tree species that dominated the local community at each location (Table S1). The litter samples were manually collected during leaf senescence. The litter from multiple individual trees of each species was pooled and dried at 60°C.

A total of 5 g single or mixed location-specific litter was incubated in each litter bag (20 × 20 cm) made of nylon mesh with different opening sizes. The bottom side of the litter bags with a 5-mm opening size was made of 1 mm nylon mesh to prevent the loss of small litter particles from the bottom. The litter bags were held in place while the bottom mesh was in intimate contact with the soil surface, and they were randomly distributed within the blocks that were established at least 20 m apart from each other. Different treatment litterbags were separated from each other by at least 50 cm. The litter bags were harvested four times at ~4 month interval.

2.3 | Mass, C and N losses from litterbags

After the litter bags were collected, they were dried to constant mass at 65°C, then cleaned litter by gently brushing off any dirt, before measuring dry mass for each constituent species and resulted in a total of 4,032 samples (n = 7 locations × 3 forest types × 4 blocks × 2 litter treatments [incubated in a mixture or monoculture] × 2 litter types [bamboo or native tree] × 3 mesh sizes × 4 harvestings). Given that changes in litter mass loss rates do not always mirror changes in nutrient cycling rates (Castro-Diez et al., 2014; Prescott & Zwikszt, 2016), we measured litter C and N contents, which allowed us to correct for soil contamination of the litter retrieved from the field. Litter C and N losses were determined in the second collection (after about 8 months of decomposition). Mass loss, C and N concentrations of the litter were determined for each sorted litter type (i.e. bamboo or native tree species) from each litterbag. This process resulted in a total of 1,008 samples (n = 7 locations × 3 forest types × 4 blocks × 2 litter types [bamboo or native tree species] × 2 litter treatment [incubated in a mixture or monoculture] × 3 mesh sizes). The initial leaf litter concentrations of phosphorus (P) and lignin were also determined for each of the 14 individual litter types from five randomly selected samples.

Samples for C and N measurements were ground with a ball mill to fine powder. Subsamples of 2 mg were analysed for C and N concentrations using an elemental analyzer (Costech ECS 4010). Litter C and N loss (%) was calculated as 100 × [(Mi × CNi) - (Mf × CNf)]/(Mi × CNi), where Mi and Mf are the initial and final litter dry mass, respectively, and CNi and CNf are the initial and final C or N concentrations (% of litter dry mass). The P concentrations in the initial litter samples were determined by an automatic chemical analyzer (Smartchem 300, AMS). Initial leaf litter lignin content was determined gravimetrically after digestion with 72% H2SO4 for 2.5 hr followed by hydrolysis with 3% H2SO4 for 4 hr.
2.4 | Statistical analysis

The annual decomposition constant $k$ was calculated using a single exponential decay model: $\ln(M_t/M_0) = kt$, where $M_0$ is the original mass of the litter, $M_t$ is the mass remaining at time $t$ and $t$ is the time in years. The $k$ values were calculated for both the single incubated litter and the individual species in the mixed species treatment at each site. When evaluating the effects of litter-mixing on N loss, we calculated the relative change in the total amount of litter N as $\left[\frac{N_{i,m} - N_{i,a}}{N_{i,a}}\right] - \left[\frac{N_{i,a} - N_{i,a}}{N_{i,a}}\right]$, where $N_{i,m}$ and $N_{i,a}$ are the initial (i), and $N_{i,m}$ and $N_{i,a}$ are the final (f) amounts of N in a particular litter type in a mixture (m) or alone (a). The degree of bamboo invasion was quantified by calculating the per cent of basal area of bamboo culms in the stand. We used a GLM ANOVA to assess the effects of litter type (bamboo vs. native tree species), litter-mixing (incubated in a mixture vs. monoculture), decomposer community (small, small + medium-sized, and all sizes), incubation habitat (incubated in pure bamboo forest, bamboo invaded and non-invaded), the locations across the climate gradient and their interactions on decomposition constant $k$, C and N loss. Linear regression analysis was conducted to test the effects of litter quality, climate variable on the decomposition constant. We used ANCOVA to test for differences in the slopes of the relationship between C and N loss either for different litter types, litter treatments, incubation habitats or different decomposer community treatments. To account for the location effect, we used MAT or MAP as covariates when doing GLM analysis, and used ANCOVA to test for differences in the slopes of the relationships between MAT or MAP and $k$ either for different incubation habitats or different decomposer community treatments. Post hoc comparisons of slopes (ANCOVA) were performed using the Tukey’s HSD (honestly significantly difference) test. All statistical analyses were conducted using IBM SPSS Statistics 21.0 (IBM SPSS Statistics Inc.).

3 | RESULTS

3.1 | Effects of litter-mixing and habitat-alteration

Bamboo leaf litters decomposed 13% faster on average than leaf litters from native tree species (Figure 2; Table 1), attributable to their higher litter quality represented by their higher N content and low lignin-to-N ratio (Tables S2 and S3). Interestingly, C loss of bamboo litter was 18.5% (35.5% vs. 41.9%) less while N loss is 79% (29.5% vs. 16.5%) greater than the paired native tree species (Figure 2). Litter decomposition in mixtures ($k = 0.70 \pm 0.02$) was 9.5% faster than that in monocultures ($k = 0.63 \pm 0.02$) regardless of litter type across all sites (Figure 2). Specifically, bamboo and native tree species litters decomposed 7.6 and 12.7%, respectively, faster in mixtures than in monocultures (Figure 3). Litters decomposition in a mixture had comparable C loss ($F_{1.896} = 0.07$, $p = 0.79$), but 28.9% more N loss ($F_{1.896} = 10.07, p < 0.01$) than that in a monoculture (Figure 2). More specifically, across all the sites, bamboo litter lost more, but insignificant ($F_{1.446} = 2.35, p > 0.05$), of its initial N when it was decomposed in mixtures (31.5%) than in monocultures (27.6%), but mixing with bamboo litter almost doubled ($F_{1.450} = 8.62, p < 0.01$) the loss of initial litter N of native tree species (20.4%) than in monocultures (12.7%; Figure 3). Furthermore, the triggered N loss of native tree litters due to mixing with bamboo litter declined with the MAT but was independent of MAP (Figure 4).

There was no incubation habitat effect on decomposition when all litter bags of different mesh sizes were pooled together (Figure 2; Table 1), and the degree of bamboo invasion did not explain the variation of $k$ values across sites (Figure S2), but surface soil C-to-N ratio was negatively correlated and N-to-P ratio was positively correlated with the $k$ value across sites (Table S3).

3.2 | Effects of decomposer organisms

The large-sized (<5 mm) decomposer community increased the average decay rate across all sites by 27 ± 6%, compared to the small-sized (<50 µm) decomposer community (Figure 2, $p < 0.001$; Table 1). However, the medium-sized decomposer community (<1 mm) reduced the decay rate by 8.0 ± 7.4%, compared to the small-sized decomposer community (Figure 2, $p < 0.001$; Table 1). The large-sized decomposer community had 13.7% more C loss and 18.9% more N loss than the small-sized decomposer community, comparable C and N losses were observed between the medium- and small-sized decomposer community (Figure 2; Table 1). The MAT and MAP together explained 35%–65% of the variance in decay rate among locations (Figure S3). The large-sized decomposer community was the most sensitive, while decomposition mediated by medium-sized invertebrates showed the least dependence on temperature and precipitation (Figure S3).

Interestingly, incubation habitat (i.e. forest types) and the body size of the decomposer community interacted with each other to affect decomposition rates (Figure 5; Table 1), with bamboo invasion decreased decay rates mediated by large-sized decomposer communities by 16%, while increased that mediated by medium-sized decomposer community by 8% (Figure 5). No interaction between the body size of the decomposer community and litter-mixing was found (Table 1), but litter-mixing accelerated decomposition by 7.2% and 7.0% when it was mediated by small- and medium-sized decomposer communities, while accelerated decomposition by 12.9% when it was mediated by large-sized decomposer community. The C loss was positively correlated with N loss, but showed significant interactions with litter type, litter-mixing, incubation habitat and completeness of the decomposer community (Figure 6), where litter-mixing lowered (Figure 6b) while habitat-alteration induced by bamboo invasion increased (Figure 6c) the regression slopes between C loss and N loss. Meanwhile, the highest regression slope between litter C loss and N loss occurred when the macrofauna were included in the decomposition process (Figure 6d).
FIGURE 2  Treatment (panels from left to right represent litter type, litter-mixing, incubation habitat, decomposer community and location) effects on annual decomposition constant, $k$ (a), nitrogen (N) loss (b) and carbon (C) loss (c). Solid circles mean the 95th and 5th percentiles, the range of each column is from 25th to 75th percentile, the short dash in each column indicates the mean, the dash in each column is the median. Significant ($p < 0.05$) values are presented in bold, and different lowercases above the x-axis denote significant difference at $p < 0.05$ level.
### Table 1

| Source of variation                              | df | k F     | p     | C loss F | p     | N loss F | p     |
|------------------------------------------------|----|---------|-------|----------|-------|----------|-------|
| Variation associated with biotic factors         |    |         |       |          |       |          |       |
| Litter type                                     | 1  | 22.2 ***|       | 38.4 ***|       | 89.9 ***|       |
| Litter-mixing                                   | 1  | 9.9 **  |       | 0.3 0.57|       | 22.6 ***|       |
| Decomposer community                            | 2  | 67.6 ***|       | 6.0 **   |       | 3.1 *    |       |
| Litter type × litter-mixing                     | 1  | 0.1 0.79|       | 5.5 *    |       | 2.3 0.13 |       |
| Decomposer community × litter type              | 2  | 1.7 0.18|       | 0.01 0.99|       | 0.1 0.95 |       |
| Decomposer community × litter-mixing            | 2  | 0.6 0.54|       | 0.1 0.90 |       | 0.4 0.67 |       |
| Variation associated with site                  |    |         |       |          |       |          |       |
| Location                                        | 6  | 257.6 ***| 107.3 ***| 104.1 ***|      |
| Incubation habitat                              | 2  | 2.1 0.13| 1.5 0.22| 0.2 0.85 |      |
| Location × incubation habitat                   | 12 | 6.3 *** | 3.0 ***| 1.9 *    |      |
| Variation associated with site or biotic        |    |         |       |          |       |          |       |
| Location × decomposer community                 | 12 | 13.8 ***| 1.7 0.07| 2.0 *    |      |
| Location × litter type                          | 6  | 13.1 ***| 13.3 ***| 13.3 *** |      |
| Location × litter-mixing                        | 6  | 2.6 * | 8.4 ***| 11.7 *** |      |
| Incubation habitat × decomposer community       | 4  | 2.8 * | 0.4 0.85| 0.8 0.55 |      |
| Incubation habitat × litter type                | 2  | 0.4 0.64| 2.7 0.07| 0.4 0.70 |      |
| Incubation habitat × litter-mixing              | 2  | 0.5 0.61| 0.2 0.79| 1.1 0.34 |      |
| Location × incubation habitat × decomposer community | 24 | 1.8 * | 1.0 0.46| 1.3 0.16 |      |
| Location × decomposer community × litter type   | 12 | 1.9 * | 1.2 0.26| 1.8 *    |      |
| Location × decomposer community × litter-mixing| 12 | 1.7 0.07| 0.8 0.61| 0.4 0.95 |      |
| Location × litter type × litter-mixing (no significant four-way interactions) | 6  | 1.4 0.20| 3.1 ** | 1.0 0.42 |      |

Note: The relative contributions of variance in decay constant (k), C and N loss associated with sites, litter type and decomposer community in a large-scale leaf litter decomposition experiment. The main factors are italicized. Analysis of variance based on GLM (see Section 2). df, degree of freedom.

*p < 0.05; **p < 0.01; ***p < 0.001.

### 3.3 Interactions between incubation habitat, decomposers and climate

Significant three-way interactions were observed among incubation habitat, the body size of the decomposer community and study location (Table 1). The sensitivities of decomposition rate in response to MAT or MAP were reduced due to bamboo invasion (Figure 7). Specifically, the reductions in climatic dependence occurred for the small- (Figure 7d) and large-sized (Figure 7g,h) decomposer communities, but not the medium-sized decomposer community (Figure 7e,f).

### 4 Discussion

Our full factorial and complete reciprocal transplanting experiment allowed us a comprehensive evaluation of how plant invasion affect litter decomposition, through both effects of litter-mixing and habitat-alteration. Our results show strong field evidence of functional diversity of decomposers, especially the presence or absence of macrofauna, in determining the effect of a woody grass invasion on litter decomposition, and reveal complex interactions among plant species changes, soil decomposer organisms and climate on C and N transformation. Contrast with the prevailing efforts of employing litter traits to predict litter decomposition altered by invasive species, this study emphasized the role of decomposer functional diversity, versus decomposers as a whole or only focusing on soil microbes, and their interactions with litter traits in regulating litter decomposition, as affected by plant invasion (see the conceptual diagram, Figure 1). Our results agree with the second hypothesis, but bamboo invasion contrastingly affected litter decomposition through litter-mixing and habitat-alteration. Where, litter-mixing stimulated decomposition irrespective of functional diversity of decomposers, litters regardless of type in the invaded site decomposed more quickly when macrofauna were excluded from
the decomposition, but more slowly as they were permitted to
access the litters. Furthermore, the climatic sensitivity of decom-
position was depressed at the invaded than non-invaded sites
when macrofauna were present. These results suggest a modu-
lating role of macrofauna on the litter decomposition under dis-
turbance of a woody grass invasion (Figure 1).

**FIGURE 3** Interactive effects between litter type and litter-
mixing on annual decomposition constant, $k$ (a), nitrogen (N) 
loss (b), and carbon (C) loss (c). Solid circles mean the 95th and
5th percentiles, the range of each column is from 25th to 75th
percentile, the short dash in each column indicates the mean, 
the dash in each column is the median. ***, ** and * denote significant 
treatment effect or interaction at $p < 0.001$, $p < 0.01$ and $p < 0.05$
levels respectively, and ns denotes no significant treatment effect

**FIGURE 4** Climatic dependence of triggered N loss of native tree litters due
to mixing with bamboo litters. X axis is (a) 
MAT, Mean annual temperature; and (b) 
MAP, Mean annual precipitation. Y axis is 
the N loss differences of litters between 
being incubated in a mixture and alone for 
native tree species

**FIGURE 5** Effects of decomposer community, that is, 
most reduced decomposer community (5 μm), medium-sized 
decomposers (1 mm) and the complete decomposer community 
(5 mm), on annual decomposition constant, $k$ (a), nitrogen (N) loss 
(b) and carbon (C) loss (c) when litters were exposed in different 
forest types (pure bamboo, non-invaded native community 
and native community invaded by bamboo). Solid circles mean the 95th 
and 5th percentiles, the range of each column is from 25th to 75th
percentile, the short dash in each column indicates the mean, 
the dash in each column is the median. ***, ** and * denote significant 
treatment effect or interaction at $p < 0.001$, $p < 0.01$ and $p < 0.05$
levels respectively, and ns denotes no significant treatment effect
FIGURE 6  Visualization of covariance analyses (ANCOVA). Analyses were based on N loss being the response variable, while C loss was covariate (shown on x-axis). Factors were litter type (a), litter treatment (b), incubation habitat (c) and decomposer communities (d). The $F$ and $p$ values are for interactive effects between factors and covariate.

FIGURE 7  Visualization of covariance analyses (ANCOVA). Analyses were based on decay rate $k$ being the response variable, while mean annual temperature (MAT) or mean annual precipitation (MAP) were covariates (shown on x-axes). Factors were incubation habitat regardless decomposer communities (a, b), or considering decomposers, that is, small (c, d), small + medium-sized (e, f) and all sizes (g, h). The $F$ and $p$ values are for interactive effects between incubation habitat and covariates, and significant interactions are presented in bold.
4.1 | Effects of litter-mixing on litter decomposition

A generalization is that litter of invasive plants decomposes faster than litter of native plant species (Arthur et al., 2012; Rothstein et al., 2004), leading to more rapid cycling of nutrients and release of C in invaded ecosystems (Prescott & Zukswert, 2016). Our results are consistent with this expectation, since bamboo litter decomposed faster than litter from native tree species (Figure 2). However, few studies have considered the effect of litter-mixing due to plant invasion, which has been shown to have a non-additive effect on decomposition (Lecerf et al., 2011; Wardle et al., 2003). Our observation of greater decomposition of mixed leaf litter from bamboo and native plant species supports the first hypothesis. This is consistent with the fact that litter mixtures are more readily decomposed than litter from a single species (Ashton et al., 2005; Handa et al., 2014; Rodgers et al., 2008), but differ from the lower mass and N loss of litter from a mixture of native and exotic invasive species in a hardwood forest (Hickman et al., 2013). The synergistic effect was attributable to differences in litter quality (Schimel & Hättenschwiler, 2007) because we detected that the relative change in the total amount of litter N between two-species mixtures and monocultures was negatively related to the difference between initial N concentration between bamboo and native tree litters (Figure S4). However, the 9.5% increase of mass loss induced by litter-mixing was mainly attributed to the accelerated N loss rather than C loss, and what is surprising is that decomposing bamboo and native tree litters in a mixture increased N release from native tree litters by 60% rather than from bamboo litter. The greater N loss of native tree litters suggests that the competition for nutrients by N-assimilation microorganisms (e.g. saprotrophic fungi) was strengthened after litter-mixing (Frey et al., 2003). This is supported by the lower N demand for decomposing each unit of C in a mixture than in a monoculture (Figure 6b), implying resource (i.e. N) complementarity. Another possible explanation is that bamboo expansion produces large amounts of litter with distinctive secondary compounds such as flavonoids (Keski-Saari et al., 2008), causing adaptation/selection of soil organisms (Chomel et al., 2016) such as ectomycorrhizal (Siqueira et al., 1991) and saprotrophic fungi (Pec et al., 2016) on litters, that lead to organisms feeding on native tree litters without phenolic, resulting in the home-field advantage (Ayres et al., 2009), and consequently faster N release.

The increased N cycling due to litter-mixing, representing approximately 0.98 g N/m², or 11% of the annual N input from leaf litter fall, could largely relieve the N limitation in the invaded ecosystem and affect the coupled soil C transformation process. However, the increased N release as a result of litter-mixing declines with MAT (Figure 4) across the climatic gradient strongly suggests that the invasion effect on nutrient availability decreased under warmer conditions. We expect that different functional groups of decomposers may play different roles in litter-mixing effect, but no interaction between functional diversity of decomposers and litter-mixing was observed despite the presence of macrofauna led to a higher positive litter-mixing effect than it was excluded (e.g. 12 vs. 7.1%), suggesting a common contribution to synergistic effect from decomposers of different trophic level.

4.2 | Effects of soil decomposer diversity on litter decomposition

Consistent with previous studies (Bradford et al., 2002; Handa et al., 2014), we found that the presence of different-sized decomposer communities in litterbags influenced decomposition across all sites. Our finding that complete decomposer community (5 mm mesh size) induced fastest litter decomposition confirmed the critical role of macrofauna on decomposition (García-Palacios et al., 2013; Handa et al., 2014) through litter consumption and indirect effect (e.g. ecological engineering, predation on medium-sized fauna), and the enhancement on decomposition (27 ± 6%) was within the range indicated in a global meta-analysis (García-Palacios et al., 2013). Also, the highest climate sensitivity of litter decomposition was observed when macrofauna were included (Figure S3), these results support our second hypothesis. However, higher decomposition rate was achieved by the small-sized (50 μm) decomposer community than medium-sized (1 mm) decomposers, which might be attributed to the accelerated reproduction of micro-organisms after the exclusion of grazers, for example, fungivores such as collembola, oribatida and nematodes (Johnson et al., 2005). These results agree with the cascading effect of ecosystem engineers and predators on decomposition (Estes et al., 2011; Hawlena et al., 2012) and the top-down control on ecosystem processes (Schmitz et al., 2010), and reveal the importance of protection on macrofauna that are vulnerable to environmental stressors (Duffy, 2003).

4.3 | Macrofauna-modulated litter decomposition altered by plant invasion

Few have studied the effect of habitat alteration caused by plant invasion on litter decomposition by reciprocal litter transplant experiment. In a mixed deciduous forest, Ashton et al. (2005) observed substantially faster decomposition in invaded sites for both invasive and non-invasive species litter, where they deployed litterbags with a mesh size of 1 mm. Here we employed litterbags of different mesh sizes (e.g. 5 mm, 1 mm and 50 μm), and we found that incubation habitat (i.e. forest type) interactively affected litter decomposition with functional diversity of decomposers. The decomposition was decelerated when macrofauna were present (5 mm) while it was accelerated when they were excluded (1 mm) from the decomposition process after plant invasion (Figure 5). These findings imply the differential role of decomposer functional groups in modulating litter decomposition under plant invasion. These reduced litter decomposition might be attributed to the community of macrofauna that was affected by invasion. Our results suggest that when we consider the role of macrofauna, the invasion of plant cannot accelerate...
litter decomposition as we anticipated, and challenges the nutrient facilitation hypothesis (Ehrenfeld et al., 2001; Holdredge & Silliman, 2010) raised by trait difference between invasive and native species (Liao et al., 2008; Prescott & Zukswert, 2016). Nevertheless, it is clear that the slowed decomposition induced by plant invasion was attributable to the nutrient competition, because the increased regression slope between C and N loss due to habitat-alteration (Figure 6c) suggests that invasion resulted in higher N demand for consuming each unit of C by decomposers, that is, decreased the nutrient use efficiency of decomposers. More importantly, macrofauna played a significant role therein because they had higher N demand for consuming each unit of C than other decomposer organisms (Figure 6d). This confirms the critical role of macrofauna in decomposition under plant invasion. An increasing evidence has demonstrated that soil fauna, especially for higher trophic groups, affects litter affinity effect (Austin et al., 2014; Milcu & Manning, 2011) or shows preferences for food resources (Wardle et al., 2004). We speculate that the much higher (more than 10 times) uptake rate of silicon of bamboo than other plant species (Cornelis & Delvaux, 2016) leads to high silicon concentration of litters, which diminishes the growth of soil animals (Frew et al., 2017), and hence impacts on litter decomposition directly by affecting C accessibility, and also indirectly by grazing on microbes. Moreover, the continuous input of high silicon litters may result in legacy effects (Kostenko et al., 2012; Suseela et al., 2016), interact with soil biota in a long term and lead to negative impact on macrofauna community and consequently slowed litter decomposition.

4.4 | Plant invasion affected the climatic dependence of litter decomposition

The most sensitive responses of decomposition to MAT and MAP when exposed to complete decomposer community (Figure S3) suggest the primary control of macrofauna on decomposition under projected climate change. More importantly, bamboo invasion reduced temperature or moisture dependence of decomposition (Figure 7), suggests that the activities of decomposers in response to climate were suppressed due to habitat-alteration, especially for the macrofauna (Figure 7g,h), hence a warmer or wetter condition cannot accelerate the decomposition as regularly (Crowther & Bradford, 2013; Crowther et al., 2016; Davidson & Janssens, 2006). This confirms our previous speculation of interaction between macrofauna and litter affinity effect, and magnifies the significance of macrofauna in modulating negative effects under warmer or wetter conditions. On the other hand, a warmer and drier climate put macrofauna at a higher extinction risk (Duffy, 2003), thus ecosystems even if there is a well-developed soil macrofauna community will face a more vulnerable response to plant invasion, given the fundamental role of macrofauna in driving litter decomposition. Nevertheless, the effects of plant invasion on litter decomposition in response to climate change depends on the combination of changes in both temperature and moisture, as the combination of warmer and drier conditions could reduce feeding activity of soil detritivores (Thakur et al., 2018) despite warming alone enhancing the activities of decomposers (Allison et al., 2010; Gillooly et al., 2001), and some areas are projected to be wetter while others to be drier (Feng et al., 2011) in the future.

In summary, we show that soil decomposer organisms of different functional groups modulated effects of plant invasion on litter decomposition, and the potential responses to climate change (Figure 1). We show that simple comparisons of trait differences between non-invasive and invasive plant species could underestimate plant invasion effects on litter decomposition when ignoring litter-mixing effect. Moreover, macrofauna played a vital role in altering the plant invasion effect, and litter decomposition was suppressed when macrofauna were included but accelerated when they were excluded from litter bags, especially under warmer and wetter conditions. These results are applicable in the case of a woody grass invasion of forests, but need to be confirmed for invasive trees in forests. Nevertheless, our results highlight that including decomposer functional groups and their interactions with litter traits in the framework would obtain a mechanistic and more reliable prediction on ecosystem functions (e.g. litter decomposition) altered by invaders, under current and future climate.

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AUTHORS’ CONTRIBUTIONS

J.L. designed the experiment and conducted the statistical analyses; J.L., S.Liu., S.Li. and Y.W. conducted the experiment and analysed the samples. J.L., S.Liu, S.Li, J.K.W., Y.W., J.W., Y.L., W.D. and S.X.C. contributed to the writing.

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DATA AVAILABILITY STATEMENT

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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