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Species Number of Invasive Plants Negatively Regulates Carbon Contents, Enzyme Activities, and Bacterial Alpha Diversity in Soil

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ABSTRACT: The leaves of multiple invasive plants can coexist and intermingle within the same environment. As species number of invasive plants increases, variations may occur in decomposition processes of invasive plants, soil nutrient contents, soil enzyme activities, and soil microbial community structure. Existing progress have predominantly focused on the ecological effects of one species of invasive plant compared to native species, with limited attention paid to the ecological effects of multiple invasive plants compared to one species of invasive plant. This study aimed to determine the differences in the effects of mono- and co-decomposition of four Asteraceae invasive plants, horseweed (*Erigeron canadensis* (L.) Cronq.), Guernsey fleabane (*E. sumatrensis* Retz.), daisy fleabane (*E. annuus* (L.) Pers.), and Canada goldenrod (*Solidago canadensis* L.), on litter decomposition responses, soil carbon contents, soil enzyme activities, and soil bacterial community structure. Species number of invasive plants did not significantly affect on the decomposition rate of mixed leaves or mixed-effect intensity of co-decomposition. Soil pH and electrical conductivity enhanced as species number of invasive plants increased. Soil carbon contents (including soluble organic carbon content and microbial carbon content), soil enzyme (including polyphenol oxidase, FDA hydrolase, and sucrase) activities, soil bacterial alpha diversity (including the OTU species, Chao1 richness, ACE richness, and Phylogenetic diversity indexes), and the number of pathways of most functional genes of soil bacterial communities closely related to decomposition processes declined as species number of invasive plants increased. Hence, soil pH and electrical conductivity significantly increased with increasing species number of invasive plants, but soil carbon contents, soil enzyme activities, soil bacterial alpha diversity, and the number of pathways of most functional genes of soil bacterial communities closely related to decomposition processes significantly reduced with growing species number of invasive plants.

KEYWORDS: Co-decomposition; co-invasion; decomposition processes; functional gene; soil bacterial community

1 Introduction

The ecological security issues triggered by biological invasions facilitated by invasive plants (IPS) have emerged as a prominent concern among ecologists currently [1–4]. The species number of IPS (S_{IPS}) presently existing in China is 515 [5]. The S_{IPS} of the Asteraceae family (92 species) is the greatest at the family level

in China [5]. It is, therefore, imperative to clarify the foremost mechanisms underpinning the successful colonization of IPS [2,4,6,7], especially of Asteraceae IPS [2,8–10].

One of the chief factors contributing to the success of IPS invasion is its capacity to interact with soil microbes through decomposition processes. This permits IPS to release numerous nutrients, thereby fluctuating soil nutrient profile (e.g., carbon) and the metabolic activity and diversity of soil microbes, which can generate a soil microenvironment that is more conducive to their further invasion [11–14]. In particular, the decomposition rate, soil carbon content, soil microbial carbon content, soil ammonium content, soil nitrate content, and soil microbial nitrogen content exhibited 116.80%, 6.86%, 34.12%, 29.68%, 16.58%, and 25.81% increase in IPS compared to native plants, respectively [15].

The successful colonization of one IPS has been demonstrated to cause fluctuations in nutrient contents, enzyme activities, and microbial community structure in soil. This, in turn, has been shown to increase the possibility of the successful colonization of other IPS in a given environment [7,16–18]. Accordingly, the co-invasion of multiple IPS is a prevalent phenomenon [11,19–21]. In particular, Asteraceae IPS can typically coexist in the same environment in Jiangsu, China, which has been shown to result in noteworthy variations in both plant community structure and soil bacterial community structure (SBC) [20,22,23]. Therefore, leaves of multiple Asteraceae IPS can coexist and intermingle in the same environment [12,23–25].

During decomposition processes, plants can release numerous components (especially carbon-containing substances). These substances have the capacity to influence soil nutrient (e.g., carbon) contents, soil enzyme activities, and SBC. Nevertheless, soil nutrient content, soil enzyme activities, and SBC, in turn, affect the decomposition processes of plants [14,23,26,27]. In particular, SBC is one of the vital regulators of decomposition rate, especially SBC plays a vital role in the initial stages of decomposition processes of plants [28–31]. Thus, decomposition processes of IPS, soil nutrient contents, soil enzyme activities, and SBC may be affected by the increase in S_{IPS} due to altered leaf quantity and quality in invaded environments following the establishment of co-invasion [24,32–34]. Thus, further research is required to examine the relationship between S_{IPS} and decomposition processes of multiple IPS, soil nutrient (e.g., carbon) contents, soil enzyme activities, as well as SBC. This can facilitate the illumination of the main mechanisms underlying the co-invasion facilitated by multiple IPS, especially with a gradient of S_{IPS}. However, extant research has predominantly concentrated on the ecological effects of one species of invasive plant in comparison to native species, with limited attention paid to the ecological effects of multiple invasive plants in comparison to one species of IPS. Additionally, there is presently a paucity of research progress in determining the relationship between S_{IPS} and decomposition processes of multiple IPS, soil nutrient (e.g., carbon) contents, soil enzyme activities, as well as SBC, especially with regard to decomposition processes.

The present study was conducted with the objective of illuminating the differences in the effects of mono- and co-decomposition of four IPS, horseweed (*Erigeron canadensis* (L.) Cronq.), Guernsey fleabane (*E. sumatrensis* Retz.), daisy fleabane (*E. annuus* (L.) Pers.), and Canada goldenrod (*Solidago canadensis* L.), on litter decomposition responses, soil carbon contents, soil enzyme activities, and SBC. This study was completed via a polyethylene-litterbag experiment with a six-month period.

We hypothesize that the decomposition rate of multiple IPS, soil carbon contents, soil enzyme activities, SBC alpha diversity, and the number of functional gene pathways of SBC closely related to decomposition processes (No-FGP) will increase as S_{IPS} increases during decomposition processes.

2 Materials and Methods

2.1 Selection of IPS

Four IPS were selected for investigation: horseweed, Guernsey fleabane, daisy fleabane, and Canada goldenrod (Figs. S1–S4). Intact and mature leaves from the adult individuals of the four IPS (more than ten individuals) were randomly collected in Zhenjiang (32.163–32.209° N, 119.456–119.529° E), Jiangsu, China, in May 2022. All of these IPS belong to the Asteraceae family, and S_{IPS} of Asteraceae family is the greatest at the family level in China [5]. The four IPS originate from America, and S_{IPS} originating from America is higher than those originating from other areas [5]. The four IPS have comparable growth patterns, with the peak growth season occurring between April and August in Jiangsu, China. They also exhibit analogous life styles, with erect herbs being a common trait. They are also found in alike environments, including wastelands, agroecosystems, and both sides of the main roads in Jiangsu, China. The four IPS have the ability to form extensive monodominant communities when they invade independently, which can decrease biodiversity in native communities [35–38]. Thus, they have been designated as harmful IPS in China. The four IPS can coexist in the same environment (Figs. S5–S15).

2.2 Experimental Design

Decomposition processes of the four IPS was replicated in a polyethylene-litterbag experiment conducted from 01 June 2022 to 02 December 2022 (~184 d) in a greenhouse at Zhenjiang (32.2061° N, 119.5128° E) at room temperature. The air-dried leaves from the four IPS were positioned in the polyethylene-litterbags (mesh size: ~0.425 mm; dimensions: 10 × 15 cm). The leaves from the four IPS were scheduled in one of the next fifteen types per polyethylene-litterbag: (1) 6 g leaves from one species of the four IPS (four types); (2) 6 g equally mixed leaves from two species of the four IPS (six types); (3) 6 g equally mixed leaves from three species of the four IPS (four types); (4) 6 g equally mixed leaves from all of the four IPS (one type). Treatments without any leaves from the four IPS were used as control. Three replicates were performed per treatment. Polyethylene-litterbags were placed into store-bought pasture soil (pH: ~6.29; organic matter content: ~324 g kg^{-1}; Shenzhibei Agr. Technol. Co., Ltd., Baishan, China) at a ~2 cm depth in planting pots (height: 16.5 cm; top diameter: 25 cm; bottom diameter: 13.4 cm; one polyethylene-litterbag per planting pot). The rationale for the application of pasture soil as culture medium is to weaken the potential for invasion experience of IPS as much as possible to become established in natural soils. Pasture soil was homogenised before the experiments were carried out. Pasture soil was not disinfected, to confirm normal existence of soil microbes. Water was applied weekly during decomposition experiment to simulate normal precipitation as well as soil moisture status in nature. The level of water application was based on the amount of precipitation in Zhenjiang. The experimental design is exemplified in Fig. 1.

According to experimental period of some studies [27,39–41], decomposition processes in this study lasted for about six months. All polyethylene-litterbags were collected after ~184 d of experimental treatment. Leaves from the four IPS in polyethylene-litterbags were slightly washed and completely air-dried to estimate decomposition variables. Soil samples were recalled at a depth of ~1 cm from polyethylene-litterbags and passed via a 2 mm-sieve to determine soil variables and SBC.
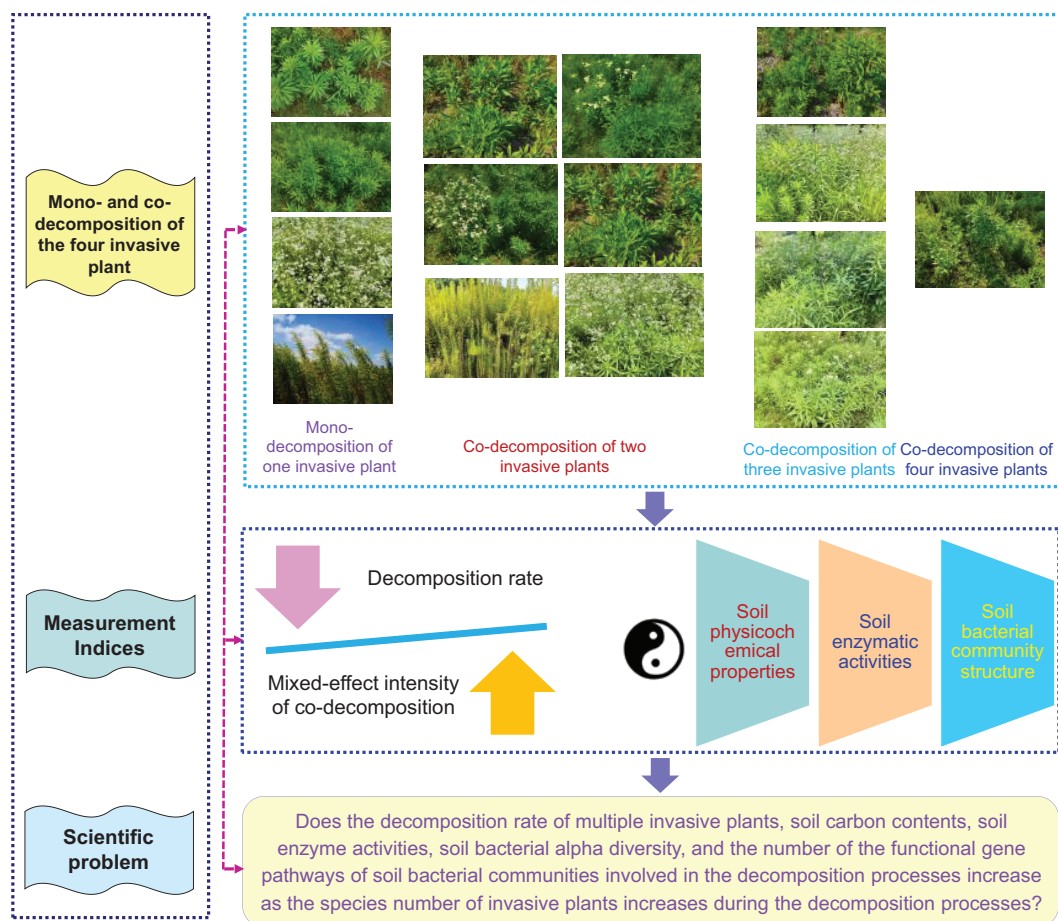
Figure 1: The chart of the experiment design

2.3 Determination of Decomposition Variables

The decomposition coefficient (k) of leaves of the four IPS, expected k of mixed leaves of the four IPS, and mixed-effect intensity of co-decomposition of leaves of the four IPS were surveyed.

The determination methods for analyzed variables closely related to decomposition processes are defined in Table S1.

2.4 Determination of Soil Variables

The pH, moisture, electrical conductivity, and the contents of total organic carbon, soluble organic carbon, and microbial carbon in soil was examined.

The activities of several enzymes closely related to soil carbon cycle were studied, including polyphenol oxidase, FDA hydrolase, cellulase, β-glucosidase, β-xylosidase, and sucrase.

The determination methods for examined soil variables are defined in Table S1.

2.5 Determination of Alpha Diversity, Beta Diversity, and Species Composition of SBC, and No-FGP

The alpha diversity, beta diversity, and species composition of SBC, and No-FGP was assayed by Co., Ltd., Guangzhou, Guangdong, China via the application of Illumina PE250 at GENE DENOVO. The primers 341F and 806R was used to amplify the V3–V4 region of the 16S rRNA genes of SBC [42,43].

The sequences were clustered into operational taxonomic units (OTUs) of ≥97% similarity using the UPARSE pipeline [44,45]. The KEGG pathway analysis of the OTUs was inferred using the Tax4Fun [46].

Soil bacterial alpha diversity was calculated using the following indices: OUT species index, Shannon diversity index [47], Simpson dominance index [48], Pielou evenness index [49], Chao1 richness index [50], ACE richness index [51], and Phylogenetic diversity index [52]. The good's coverage index was also evaluated to indicate the coverage level of the sample library [53].

Correlations in soil bacterial beta diversity were appraised using the weighted UniFrac algorithm [52] through principal coordinates analysis (PCoA) [54] and nonmetric multidimensional scaling (NMDS) [55].

2.6 Statistical Analysis

Statistical analysis of differences in the values of measured variables between various treatments was conducted using the Tukey multiple comparison test. Correlation between the values of measured variables and S_{IPS} was estimated using the correlation analysis judged by the Pearson's coefficient. The path analysis was employed to estimate the influences of the values of measured variables on the decomposition coefficient as well as the influences of S_{IPS} on the values of measured variables judged by the path coefficient (i.e., the standardized regression coefficient). Significance level was set at $p \leq 0.05$. Statistical analyses were made using IBM SPSS Statistics 26.0.

3 Results

3.1 Differences in Decomposition Variables

There were no differences between the decomposition coefficients between any of the mixes of plant litter from a single species to the four mixed species ($p > 0.05$) (data not shown). No close correlations were detected between the decomposition coefficient of the four IPS and S_{IPS} ($p = 0.325$; Table 1).

Table 1: Correlations (r) between the decomposition coefficient, soil variables, soil bacterial alpha diversity, and species number of invasive plants (S_{IPS})

		S_{IPS}			S_{IPS}			S_{IPS}
Decomposition coefficient	r	0.150	Soil polyphenol oxidase activity	r	−0.388**	Shannon diversity index	r	0.080
	p	0.325		p	**0.008**		p	0.600
Soil pH	r	0.448**	Soil FDA hydrolase activity	r	−0.504***	Simpson dominance index	r	0.271
	p	**0.002**		p	**<0.001**		p	0.072
Soil moisture	r	0.267	Soil cellulase activity	r	−0.197	Pielou evenness index	r	0.244
	p	0.077		p	0.195		p	0.106
Soil electrical conductivity	r	0.480***	Soil β-glucosidase activity	r	−0.085	Chao1 richness index	r	−0.344*
	p	**<0.001**		p	0.579		p	**0.021**
Total soil organic carbon content	r	0.189	Soil β-xylosidase activity	r	0.272	ACE richness index	r	−0.324*
	p	0.214		p	0.071		p	**0.030**
Soil soluble organic carbon content	r	−0.368*	Soil sucrase activity	r	−0.294*	Phylogenetic diversity index	r	−0.581***
	p	**0.013**		p	**0.050**		p	**<0.001**
Soil microbial carbon content	r	−0.355*	OTU species index	r	−0.333*			
	p	**0.017**		p	**0.025**			

Note: *, **, and *** indicate statistically significant differences at the 0,05, 0.01, and 0.001 probability levels, respectively. $p \leq 0.05$ are shown in bold.

The observed decomposition coefficient was similar to the expected decomposition coefficient for co-decomposition of the four IPS independent of S_{IPS} ($p > 0.05$) (data not shown). There were no significant differences in mixed-effect intensity of co-decomposition of the four IPS independent of S_{IPS} ($p > 0.05$) (data not shown). The mixed-effect intensity of co-decomposition of the four IPS independent of S_{IPS} was slightly greater than zero (data not shown).

3.2 Differences in Soil Variables

Soil pH under co-decomposition of two IPS, co-decomposition of three IPS, and co-decomposition of four IPS was greater than that under control ($p < 0.05$; Fig. 2a). Soil pH was positively related to S_{IPS} ($p = 0.002$; Table 1).
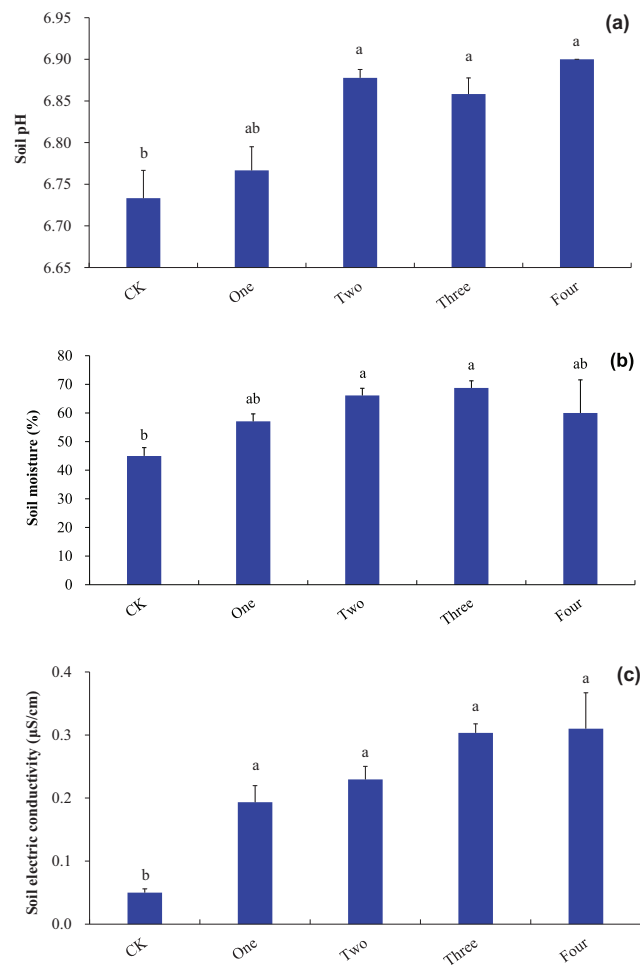

Figure 2: Soil physicochemical properties ((**a**), soil pH; (**b**), soil moisture; (**c**), soil electrical conductivity). Bars (means and SE) with different letters indicate statistically significant differences at 0.05 probability ($p < 0.05$). Abbreviation: CK, control ($n = 3$); One, mono-decomposition of one invasive plant ($n = 12$); Two, co-decomposition of two invasive plants ($n = 18$); Three, co-decomposition of three invasive plants ($n = 12$); Four, co-decomposition of four invasive plants ($n = 3$)

Soil moisture under co-decomposition of two IPS and co-decomposition of three IPS was superior than that under control ($p < 0.05$; Fig. 2b). No strong correlations were found between soil moisture and S_{IPS} ($p = 0.077$; Table 1).

The decomposition of the four IPS independent of S_{IPS} increased soil electrical conductivity compared to control ($p < 0.05$; Fig. 2c). Soil electrical conductivity was positively related to S_{IPS} ($p < 0.001$; Table 1).

Co-decomposition of two IPS decreased the contents of total organic carbon, soluble organic carbon, and microbial carbon in soil compared to control ($p < 0.05$; Fig. 3a–c). Total soil organic carbon content under co-decomposition of two IPS was less than that under mono-decomposition of one IPS, co-decomposition of three IPS, and co-decomposition of the four IPS ($p < 0.05$; Fig. 3a). The contents of soluble organic carbon and microbial carbon in soil under the co-decomposition of two IPS were lesser than those under the mono-decomposition of one IPS ($p < 0.05$; Fig. 3b,c). No strong correlations were detected between total soil organic carbon content and S_{IPS} ($p = 0.214$; Table 1). The contents of soluble organic carbon ($p = 0.013$) and microbial carbon ($p = 0.017$) in soil were negatively related to S_{IPS} (Table 1).
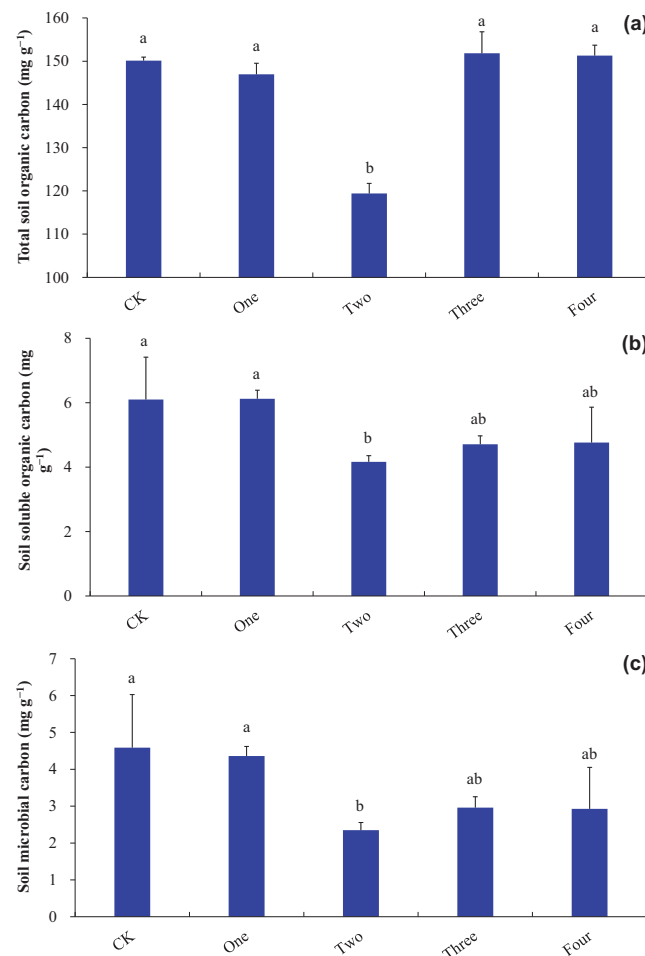
Figure 3: Soil carbon contents ((**a**), total soil organic carbon; (**b**), soil soluble organic carbon; (**c**), soil microbial carbon). Bars (means and SE) with different letters indicate statistically significant differences at 0.05 probability ($p < 0.05$). Abbreviations have the same meanings as presented in Fig. 2

Co-decomposition of three IPS and co-decomposition of the four IPS decreased soil FDA hydrolase activity compared to control ($p < 0.05$; Fig. 4b). Soil FDA hydrolase activity under the co-decomposition of three IPS was lesser than that under the mono-decomposition of one IPS and co-decomposition of two IPS ($p < 0.05$; Fig. 4b). Soil FDA hydrolase activity was negatively related to S_{IPS} ($p < 0.001$; Table 1).
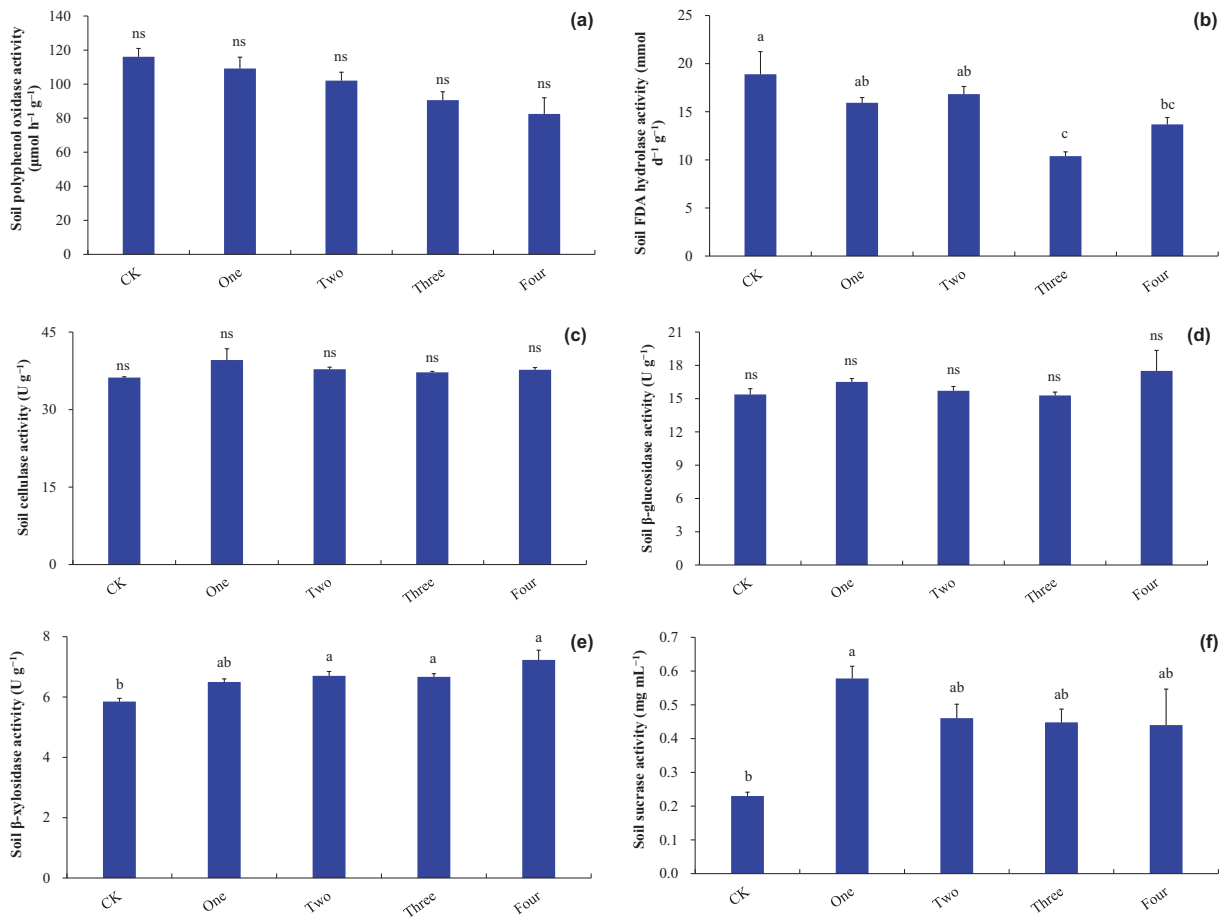
Figure 4: Soil enzyme activities ((**a**), soil polyphenol oxidase activity; (**b**), soil FDA hydrolase activity; (**c**), soil cellulase activity; (**d**), soil β-glucosidase activity; (**e**), soil β-xylosidase activity; (**f**), soil sucrase activity). Bars (means and SE) with different letters indicate statistically significant differences at 0.05 probability ($p < 0.05$). "ns" indicate no statistically significant differences at 0.05 probability ($p > 0.05$). Abbreviations have the same meanings as presented in Fig. 2

Co-decomposition of the four IPS independent of S_{IPS} increased soil β-xylosidase activity compared to control ($p < 0.05$; Fig. 4e). No robust correlations were found between soil β-xylosidase activity and S_{IPS} ($p = 0.071$; Table 1).

Mono-decomposition of one IPS increased soil sucrase activity compared to control ($p < 0.05$; Fig. 4f). Soil sucrase activity was negatively related to S_{IPS} ($p = 0.05$; Table 1).

The decomposition of the four IPS independent of S_{IPS} did not significantly affect soil polyphenol oxidase activity, soil cellulase activity, and soil β-glucosidase activity compared to control ($p > 0.05$; Fig. 4a,c,d). Soil polyphenol oxidase activity was negatively related to S_{IPS} ($p = 0.008$; Table 1). No strong correlations were observed between soil cellulase activity ($p = 0.195$), soil β-glucosidase activity ($p = 0.579$), and S_{IPS} (Table 1).

3.3 Differences in SBC Alpha Diversity

The decomposition of the four IPS independent of S_{IPS} decreased the OTU species, ACE richness, and Phylogenetic diversity indexes of SBC compared to control ($p < 0.05$; Fig. 5a,f,g). Co-decomposition of the four IPS independent of S_{IPS} decreased the Chao1 richness index of SBC compared with control

($p < 0.05$; Fig. 5e). The OTU species ($p = 0.025$), Chao1 richness ($p = 0.021$), ACE richness ($p = 0.030$), and Phylogenetic diversity ($p < 0.001$) indexes of SBC were negatively related to S_{IPS} (Table 1).
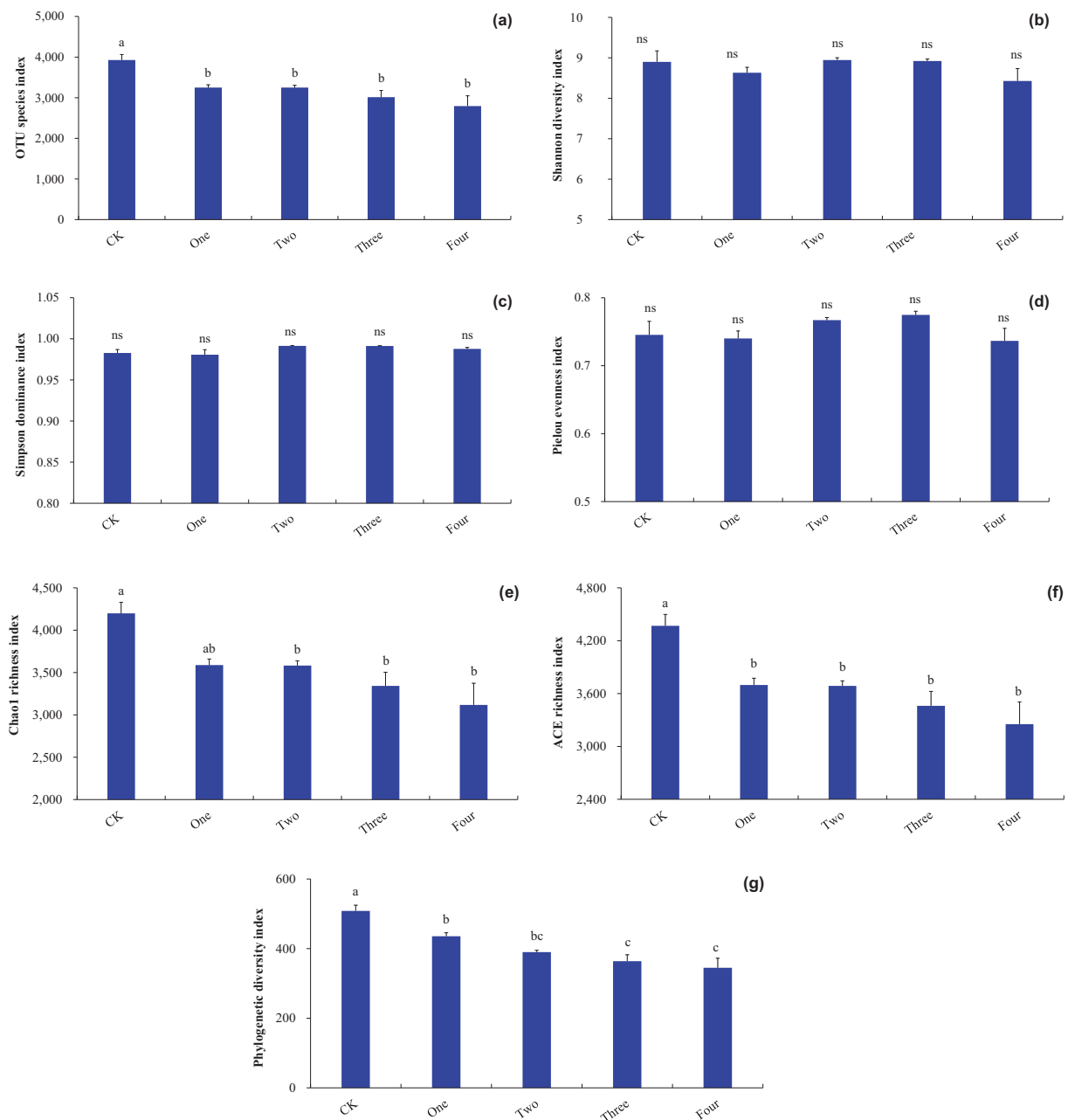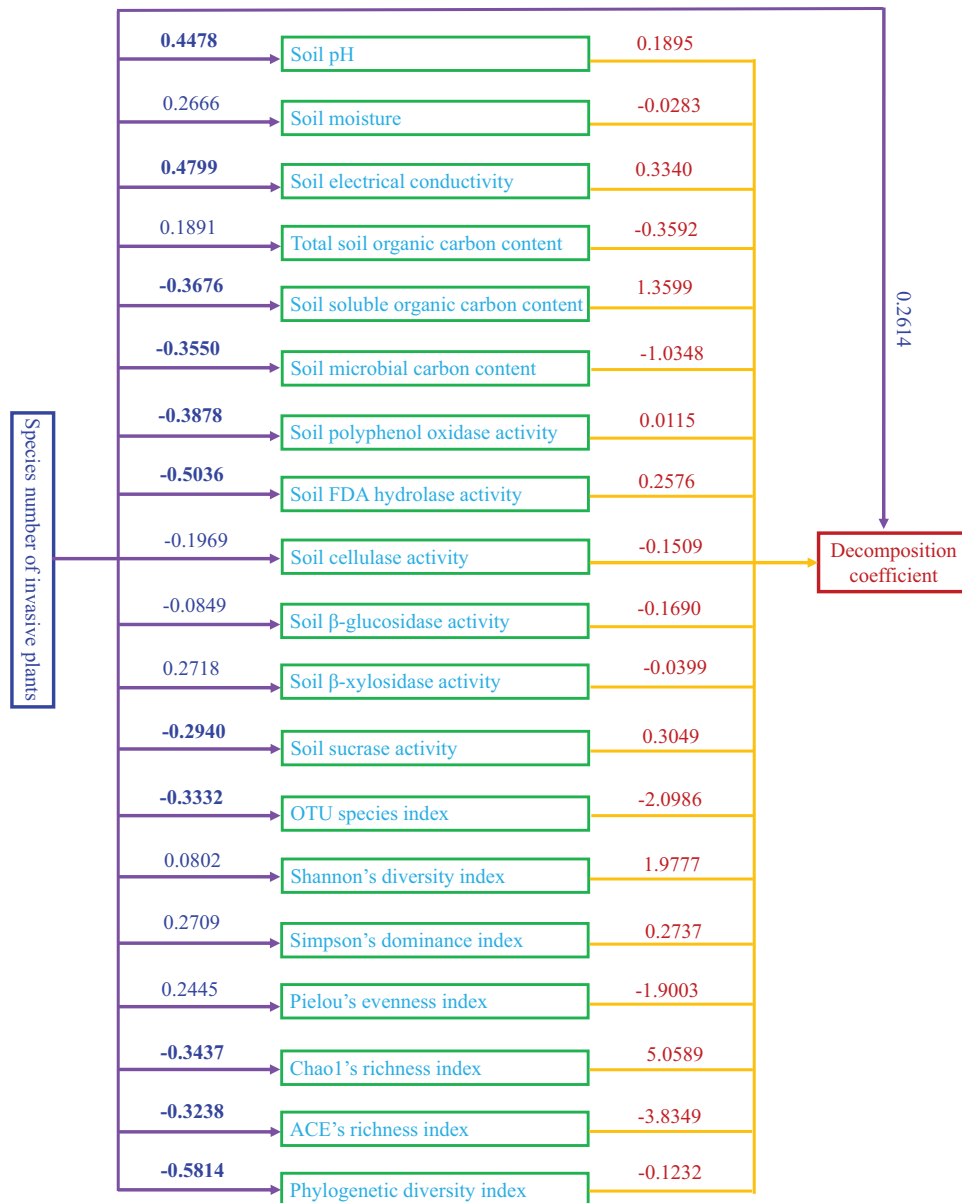

Figure 5: Soil bacterial alpha diversity ((**a**), OTU species index; (**b**), Shannon diversity index; (**c**), Simpson dominance index; (**d**), Pielou evenness index; (**e**), Chao1 richness index; (**f**), ACE richness index; (**g**), Phylogenetic diversity index). Bars (means and SE) with different letters indicate statistically significant differences at 0.05 probability ($p < 0.05$). "ns" indicates no statistically significant differences at 0.05 probability ($p > 0.05$). Abbreviations have the same meanings as presented in Fig. 2

The decomposition of the four IPS independent of S_{IPS} did not significantly affect the Shannon diversity ($p = 0.600$), Simpson dominance ($p = 0.072$), and Pielou evenness ($p = 0.106$) indexes of SBC compared

to control ($p > 0.05$; Fig. 5b–d). No robust correlations were observed between the Shannon diversity ($p = 0.600$), Simpson dominance ($p = 0.271$), and Pielou evenness ($p = 0.244$) indexes of SBC and S_{IPS} (Table 1).

3.4 Influences of Soil Variables and SBC Alpha Diversity on the Decomposition Coefficient and Influences of S_{IPS} on the Decomposition Coefficient, Soil Variables, and SBC Alpha Diversity

The S_{IPS} posed significantly positive influences on soil pH and electrical conductivity ($p < 0.05$; Fig. 6).

Figure 6: Schematic diagram of path analysis. Blue numbers represent influences of species number of invasive plants on the decomposition coefficient, soil variables, and soil bacterial alpha diversity. Fuchsia numbers represent influences of soil variables and soil bacterial alpha diversity on the decomposition coefficient. Positive values indicate positive influences, while negative values indicate negative influences. The stronger the influence, the greater the deviation from 0; and *vice versa*. $p \leq 0.05$ are shown in bold

The S_{IPS} caused significantly negative influences on soluble organic carbon content, microbial carbon content, polyphenol oxidase activity, FDA hydrolase activity, and sucrase activity in soil, and the OTU species, Chao1 richness, ACE richness, and Phylogenetic diversity indexes of SBC ($p < 0.05$; Fig. 6).

3.5 Differences in SBC

The mean value of the good coverage indices of SBC across all samples was ~0.988. The SBC beta diversity under the decomposition of the four IPS independent of S_{IPS} showed obvious differences with control based on weighted UniFrac distances (Figs. S16 and S17). There were marked differences in SBC beta diversity between mono-decomposition of one IPS and the co-decomposition of the four IPS independent of S_{IPS} based on weighted UniFrac distances (Figs. S16 and S17).

At the class level, the decomposition of the four IPS independent of S_{IPS} improved the relative abundance of Alphaproteobacteria and Actinobacteria, but apparently failed the relative abundance of Gammaproteobacteria compared to control (Fig. S18). At the order level, the decomposition of the four IPS independent of S_{IPS} increased the relative abundance of Sphingomonadales, Cytophagales, Propionibacteriales, Gemmatimonadales, and Saccharimonadales compared to control (Fig. S19). At the family level, the decomposition of the four IPS independent of S_{IPS} increased the relative abundance of Sphingomonadaceae, Microscillaceae, Sphingobacteriaceae, Nocardioidaceae, and Gemmatimonadaceae, but declined the relative abundance of Enterobacteriaceae compared to control (Fig. S20).

At the class level, the prevalence of Blastocatellia, Acidobacteriae, and Gammaproteobacteria was declined, but the prevalence of Planctomycetes, Saccharimonadia, Gemmatimonadetes, Actinobacteria, and Alphaproteobacteria was enhanced under the decomposition of the four IPS independent of S_{IPS} compared to control (Fig. S21). At the order level, the prevalence of Burkholderiales and Xanthomonadales was decreased, but the prevalence of Micrococcales, Saccharimonadales, Gemmatimonadales, Propionibacteriales, Cytophagales, and Sphingomonadales was improved under the decomposition of the four IPS independent of S_{IPS} compared to control (Fig. S22). At the family level, the prevalence of Enterobacteriaceae was decreased, but the prevalence of Pseudonocardiaceae, Nocardioidaceae, Microscillaceae, and Sphingomonadaceae was increased under the decomposition of the four IPS independent of S_{IPS} compared to control (Fig. S23).

3.6 Differences in No-FGP

There were sixty-two No-FGP that reached significant levels between different treatments in this study (Table S2).

The number of pathways of most functional genes of SBC closely related to decomposition processes reduced with increasing S_{IPS} (Table S2).

Correlations between No-FGP and S_{IPS} that reached significant levels were all negatively related ($p < 0.05$; Table S3).

4 Discussion

Multiple IPS can cooccur in the same environment [11,19,22,36]. Thus, leaves of multiple IPS can coexist in the same environment and then be degraded collectively. Hence, it is expected that the decomposition rate may be influenced by the differing characteristics of mixed leaves from multiple IPS, primarily due to the divergent quantities of exuded materials and the major components. However, there is currently no consensus on the relationship between the decomposition rate of mixed leaves from multiple IPS and S_{IPS}. Earlier studies have revealed that co-decomposition of two or more IPS can result in either a synergistic

effect [12,56–58] or an antagonistic effect [23,59–61], depending largely on the nature of interspecific facilitation or interspecific interference. In this study, the neutral effects of co-decomposition of the four IPS on the decomposition rate of mixed leaves or mixed-effect intensity of co-decomposition may be qualified to the comparable percentage of soluble substances and recalcitrant substances in the leaves from the four IPS. In other words, the quality of the leaves from the four IPS may be comparable. This phenomenon may be ascribed to the fact that they are all Asteraceae species [5], which originate from America [5], and have very similar growth patterns, lifestyles, and environments. Thus, the proportion of soluble substances and recalcitrant substances in the leaves of one IPS and mixed leaves from the four IPS was comparable. This finding may also be qualified to the moderately short period of time in this study, during which the differences in the decomposition rate of the leaves from a single IPS and mixed leaves from the four IPS have not yet manifested themselves. This result validates the earlier findings that the co-decomposition of multiple plants results in a neutral effect [23,57,62,63]. Thus, contrary to expectations, S_{IPS} does not appear to be a significant factor influencing the decomposition rate of mixed leaves or the mixed-effect intensity of co-decomposition.

In general, IPS can change soil physicochemical properties through the exudation of secondary compounds during decomposition processes [9,64–66]. In this study, the positive regulatory effects of S_{IPS} on soil pH may be attributed to the secretion of alkaline components or other components that may have an alkaline effect, e.g., anions, during decomposition processes [64,65,67,68]. The enhanced soil alkalization with increasing S_{IPS} may be attributed that there may a negative relationship between soil pH and organic carbon content [69,70]. Similarly, the positive regulatory effects of S_{IPS} on soil electrical conductivity can be ascribed to elevated ion contents in soil resulting from decomposition processes of the four IPS [71–74].

Usually, IPS may modify soil carbon contents through the exudation of carbonaceous materials during decomposition processes. In general, IPS has the potential to increase soil carbon contents by growing the input of carbon into ecosystems. This process, known as soil carbon sequestration, can be facilitated by IPS [67,75–77]. Contrary to the expected result, the negative regulatory effects of S_{IPS} on the contents of soluble organic carbon and microbial carbon in soil may be attributed to accelerated carbon metabolism of soil microorganisms as S_{IPS} increased. The negative regulatory effects of S_{IPS} on the contents of soluble organic carbon and microbial carbon in soil may also be attributed to increased soil pH and conductivity, which can lead to ion competition and therefore inhibit microbial activity [69,70]. Thus, as S_{IPS} increases, the probability of carbon being released from soil to atmosphere also increases probably. This proposes that IPS may play a more significant role in carbon emission than was formerly thought, rather than functioning as a carbon sink. Thus, the co-invasion facilitated by multiple IPS (particularly the co-invasion facilitated by two IPS) may contribute to climate change by reducing the contents of soluble organic carbon and microbial carbon in soil via decomposition processes. It is, therefore, imperative to minimize the co-invasion recruited by multiple IPS as early as possible to lessen or even prevent further carbon release from the soil through invasion process mediated by IPS. It should be noted that IPS affect carbon cycle in ways other than through decomposition processes, including growth. It is, therefore, evident that further comprehensive analysis is required to gain a more comprehensive explanation for the overall impacts of IPS on the carbon cycle.

Additionally, IPS has been detected to affect soil enzyme activities [78–81] and SBC alpha diversity [82–85] through the release of nutrients (particularly nitrogenous substances) during decomposition processes. Contrary to the expected result, the negative regulatory effects of S_{IPS} on soil polyphenol oxidase capability, FDA hydrolytic capability, and sucros hydrolytic capability may be qualified to a decrease in nutrient availability level in soil and/or an increased metabolic rate of microbes during decomposition processes of the four IPS. In addition, it is expected that mixed leaves from multiple IPS will deliver a greater variety and more complex amount of organic matter to the soil subsystem through decomposition processes. This will result in greater biochemical diversity and the increased niches of available substrates,

which will typically support the growth of a wider variety of SBC [26,86–88]. However, there may be a negative regulatory effect of S_{IPS} on SBC alpha diversity, especially the OTU species, Chao1 richness, ACE richness, and Phylogenetic diversity. This finding has already been detected in other studies [82–84,89]. Similarly, there may be a negative regulatory effect of S_{IPS} on the number of pathways of the majority of functional genes of SBC closely related to decomposition processes. Thus, the alpha diversity and No-FGP are largely influenced by the identity rather than the diversity of IPS [23,32,88,90]. The observed decrease in the alpha diversity and No-FGP with increasing S_{IPS} may be attributed to concomitant increases in soil pH and electrical conductivity. However, soil pH [91–93] and electrical conductivity [91] are thought to have significant (typically negative) effects on SBC diversity and abundance, largely by altering absorption and use of resources metabolized by SBC. Therefore, it is imperative to control the co-invasion mediated by multiple IPS as early as possible, especially in agroecosystems, wastelands, and both sides of the main roads, to alleviate or even prevent a decline in soil enzyme activities, SBC alpha diversity, and the number of pathways of functional genes of SBC closely related to decomposition processes.

It has been shown that IPS can create a favorable soil microenvironment by altering SBC, which can accelerate the subsequent invasion process via plant-soil feedback [27,94–96]. The decomposition of the four IPS independent of S_{IPS} can significantly influence SBC beta diversity and the relative abundance of specific SBC' taxa as well as lead to the emergence of numerous dominant SBC. Thus, the decomposition of the four IPS independent of S_{IPS} can significantly alter SBC by altering specific SBC' taxa. The altered relative abundance of specific SBC' taxa, in addition to the up-regulated predominant SBC and other down-regulated predominant SBC may be attributed to the selective facilitation or suppression of selection induced by the altered quantity, type, and complexity of organic matter in soil [23,66] under the decomposition of the four IPS independent of S_{IPS}, particularly decomposition processes of mixed leaves from multiple IPS.

However, the experimental design operated in this study has a few insufficiencies. For instance, the composition, especially the contents of recalcitrant components (those that decomposed slower) and soluble components (those that decomposed faster), in the leaves from multiple IPS were not subjected to investigation. Furthermore, decomposition processes of the native plants did not evaluate in this study. The mesh size of the used polyethylene litterbags may affect the access of decomposers (especially microbes) as well as their metabolic activity, which in turn may affect the results. Thus, the experimental design needs to be further enhanced. Precisely, future studies still need to be executed to determine the composition in leaves from multiple IPS as well as the differences in litter decomposition responses, soil physicochemical properties, soil enzyme activities, and SBC between native plants and mono- and co-decomposition of multiple IPS. This can facilitate the acquisition of more comprehensive data on the effects of mono- and co-decomposition of multiple IPS with a gradient of species number on the litter mass loss, soil carbon contents, soil enzyme activities, and SBC.

5 Conclusions

This study has examined the effects of mono- and co-decomposition of four Asteraceae invasive plants on soil carbon contents, soil enzyme activities, and SBC. Specifically, S_{IPS} recruits a negative influence on soil carbon contents (including soluble organic carbon content and microbial carbon content), soil enzyme activities (including polyphenol oxidase activity, FDA hydrolase activity, and sucrase activity), SBC alpha diversity (including the OTU species, Chao1 richness, ACE richness, and Phylogenetic diversity indexes), and the number of pathways of most functional genes of SBC involved in decomposition processes.

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