

## Article

# Do the Leaves of Multiple Invasive Plants Decompose More Easily than a Native Plant's under Nitrogen Deposition with Different Forms?

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**Abstract:** This study aimed to clarify the differences in the decomposition rates, soil carbon and nitrogen contents, soil enzyme activities, and the structure of the soil bacterial community between the four Asteraceae invasive plants (AIPs), *Bidens pilosa* L., *Conyza canadensis* (L.) Cronq., *Solidago canadensis* L., and *Symphytotrichum subulatum* (Michx.) G.L. Nesom, and the native plant *Pterocypsela laciniata* (Houtt.) Shih under the artificially modeled nitrogen with four forms (including nitrate, ammonium, urea, and the mixed nitrogen forms with an equal mixture of three individual nitrogen forms). The mixed nitrogen forms significantly increased the decomposition rate of the four AIPs and *P. laciniata*. The positive effects of the mixed nitrogen forms on the decomposition rate of the four AIPs and *P. laciniata* were obviously greater than those of individual nitrogen forms. Nitrogen with four forms visibly up- or down-regulated the dominant role of predominant soil bacterial biomarkers, and significantly increased the species number, richness, and phylogenetic diversity of the soil bacterial community, as well as the number of most of the functional gene pathways of the soil bacterial communities involved in the decomposition process. The decomposition rate of the four AIPs was similar to that of *P. laciniata*. The leaves of *C. canadensis* decomposed more easily than those of *S. subulatum*. The decomposition process of the four AIPs caused remarkable changes in the relative abundance of several taxa of the soil bacterial community and soil bacterial beta diversity, and caused apparent up- or down-regulation in the dominant role of predominant soil bacterial biomarkers and the number of several functional gene pathways of the soil bacterial communities involved in the decomposition process.

**Keywords:** Asteraceae; decomposition process; decomposition rate; soil bacterial community; soil enzyme activities



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## 1. Introduction

Invasive plants have caused serious ecological risks to native ecosystems, and invaders can especially lead to biodiversity loss [1–4]. The number of species of invasive plants in China is 515, and 92 species of these invasive plants (~17.86%) are in the Asteraceae family [5,6]. Therefore, elucidating the key mechanisms underlying the successful colonization of invasive plants, especially the Asteraceae invasive plants (hereafter abbreviated as AIPs) [7–10], has become one of the key scientific issues in the research field of invasion ecology at present.

The successful colonization of invasive plants may be due to the fact that invasive plants may release a variety of nutrients during the decomposition process and then alter soil physicochemical properties (especially soil acidity and soil nutrient content, etc.) as well as the metabolic activity and diversity of soil microorganisms, thereby creating a soil microenvironment more conducive to their further invasion [11–14]. More importantly, invasive plants may shed more leaves and/or their shed leaves may decompose more rapidly than those of native plants, which may provide more nutrients for the metabolic activity and diversity of soil microorganisms (especially the decomposers), thereby facilitating the successful colonization of invasive plants [15–18].

The decomposition process of plant species (including invasive plants) can be influenced by the deposition of atmospheric nitrogen (hereafter abbreviated as D-AN). In particular, D-AN contains numerous nitrogen components, and the ratio of different nitrogen components in D-AN is expected to change due to the increased intensity and frequency of anthropological activities, as well as the progressive changes in the energy policy and energy mix in China in recent years [19–22].

However, D-AN may alter soil physicochemical properties and soil microbial communities, as well as the decomposition rate of invasive plants [23–26]. The changes in the ratio of different nitrogen components in D-AN in China may also alter the effects of invasive plants on the soil environment (e.g., soil physicochemical properties and soil microbial communities), especially through the decomposition processes. Thus, evaluating the effects of the decomposition processes of invasive plants on the soil environment (e.g., soil physicochemical properties and soil microbial communities) is essential to elucidate the key mechanisms underlying the successful colonization of invasive plants (particularly AIPs), especially under D-AN with different components. However, current research in this area is limited.

This study aimed to clarify the differences in the decomposition rates, soil carbon and nitrogen contents, soil enzyme activities, and the structure of the soil bacterial community (hereafter abbreviated as SBCS) between the four Asteraceae AIPs, *Bidens pilosa* L., *Conyza canadensis* (L.) Cronq., *Solidago canadensis* L., and *Symphytotrichum subulatum* (Michx.) G.L. Nesom, and the Asteraceae native plant *Pterocypsela laciniata* (Houtt.) Shih under D-AN with different components (including nitrate, ammonium, urea, and the mixed nitrogen forms with an equal mixture of three individual nitrogen forms). This study was conducted using a seven-month-long polyethylene-litterbag experiment.

This study investigated the following questions: (1) Can D-AN increase the decomposition rates of the four AIPs and *P. laciniata*, soil carbon and nitrogen contents, soil enzyme activities, and soil bacterial alpha diversity (hereafter abbreviated as SBAD)? (2) Do the mixed nitrogen forms exert a stronger positive effect on the decomposition rates of the four AIPs and *P. laciniata* than individual nitrogen forms? (3) Is the decomposition rate of the four AIPs greater than that of *P. laciniata*? (4) Does the decomposition of the four AIPs increase soil carbon and nitrogen contents, soil enzyme activities, and SBAD compared to *P. laciniata*?

## 2. Materials and Methods

### 2.1. Selection of Invasive Plants

In this study, *B. pilosa*, *C. canadensis*, *S. canadensis*, and *S. subulatum* were selected as the invasive plants. All of these invasive plants are AIPs, and the species number of AIPs in China is larger than that of invasive plants belonging to other families [5,6]. The four AIPs originated from the Americas, and the species number of invasive plants originating from the Americas in China is larger than that of those originating from other continents and/or countries [5,6]. The four AIPs have similar life cycles (e.g., their peak growth period is usually from ~April to ~August in southern Jiangsu, China), life styles (i.e., upright herbs), and growing environments (e.g., the farmland edges, wastelands, and areas near major roads in southern Jiangsu, China, etc.). The four AIPs can often create large areas of dominant communities in southern Jiangsu, China, and then lead to biodiversity

loss [27,28], and the four AIPs have been recognized as notorious AIPs in China. The regions in China where the four AIPs have been established are also among the regions most severely affected by D-AN [19–22].

## 2.2. Selection of Native Plant

In this study, *P. laciniata* was chosen as the native plant. In particular, *P. laciniata* and the four AIPs mentioned above were the Asteraceae species. Furthermore, the life cycles, life styles, and growing environments of *P. laciniata* were similar to those of the four AIPs mentioned above. More importantly, *P. laciniata* can often coexist with one or more of the four AIPs mentioned above in the same habitat, especially in farmland edges, wastelands, and areas near major roads in southern Jiangsu, China, etc.

## 2.3. Experimental Design

The leaves of the four AIPs mentioned above and those of *P. laciniata* were harvested in Zhenjiang (32.1633–32.2092° N, 119.4565–119.5293° E), southern Jiangsu, China, in May 2022. Zhenjiang has a humid subtropical monsoon climate. The annual mean temperature of Zhenjiang in 2022 was ~17.1 °C, and the monthly mean temperature reached a maximum of ~28.1 °C in July and decreased to a minimum of ~3.7 °C in January [29]. The annual precipitation of Zhenjiang in 2022 was ~1164.1 mm, and the monthly mean precipitation reached a maximum of ~432.1 mm in July and decreased to a minimum of ~2.7 mm in December [29]. The annual sunshine duration of Zhenjiang in 2022 was ~1909.0 h, and the monthly mean sunshine duration reached a maximum of ~208.2 h in December and decreased to a minimum of ~125.9 h in August [29].

The decomposition of the four AIPs mentioned above and that of *P. laciniata* was mimicked by a seven-month-long polyethylene-litterbag experiment from 1 September 2022 to 1 April 2023 in a greenhouse at Jiangsu University (32.2061° N, 119.5128° E), Zhenjiang. The air-dried leaves of the four AIPs mentioned above and those of *P. laciniata* were packed into the polyethylene-litterbags (mesh size: ~0.425 mm; dimensions: 10 × 15 cm). Specifically, 6 g of air-dried leaves per species was placed in each polyethylene-litterbag. The polyethylene-litterbags were then buried in store-bought pasture soil (manufacturer: Lanyue Sci. & Technol. Co., Ltd., Suzhou, China; 3 kg/planting basin) at a depth of ~2 cm in planting basins (height: ~16.5 cm; upper diameter: ~21.5 cm; lower diameter: ~13.4 cm) with one polyethylene-litterbag per planting basin. The reason for using pasture soil as the culture substrate in the polyethylene-litterbag experiment was to minimize or even eliminate the possible invasion experience of AIPs, as well as the possible contamination history of D-AN in the natural soils. The pasture soil was not sterilized to allow for the normal presence of soil microorganisms. The polyethylene-litterbags per species were all treated with artificially modeled D-AN with four forms, nitrate (potassium nitrate; inorganic nitrogen), ammonium (ammonium chloride; inorganic nitrogen), urea (organic nitrogen), and the mixed nitrogen forms (nitrate/ammonium/urea = 1:1:1), at 5 g N m<sup>-2</sup> yr<sup>-1</sup> with sterile distilled water as the control (0 g N L<sup>-1</sup>). The content of D-AN in four forms was similar to the actual content (i.e., 5 g N m<sup>-2</sup> yr<sup>-1</sup>) of natural D-AN components in southern Jiangsu, China [19–22]. The proportions of three individual nitrogen forms in the mixed nitrogen forms were similar to the actual proportions (i.e., mixed in equal proportions) of natural D-AN components in southern Jiangsu, China [30–32]. Three polyethylene-litterbags were used per treatment.

All litter bags were harvested after ~210 d of the experimental treatment. The leaves of the four AIPs mentioned above and those of *P. laciniata* in polyethylene-litterbags were lightly washed and thoroughly air-dried to assess the decomposition rate. The soil samples were also collected within 1 cm of polyethylene-litterbags and passed through a 2 mm sieve to analyze soil physicochemical properties and enzyme activities, and SBCS.

#### 2.4. Determination of the Decomposition Rate

The decomposition rate of the four AIPs mentioned above and *P. laciniata* was determined using the decomposition coefficient ( $k$ ) as follows [33]:

$$X_t = X_0 \times e^{-kt}$$

where  $X_0$  and  $X_t$  are the dry weights of the leaves at the beginning and the end of the decomposition at time  $t$ , respectively.

#### 2.5. Determination of Soil Physicochemical Properties and Enzyme Activities

The pH, moisture, and electrical conductivity in soils were determined in situ using digital soil acidity, moisture, and electrical conductivity meters, respectively (code: ZD-06 & ZD-EC; manufacturer: ZD Instrument Co., Ltd., Taizhou, China) [12,24].

Soil organic carbon and microbial carbon contents, as well as organic nitrogen and microbial nitrogen contents, were determined using the chloroform fumigation method [34]. Soil nitrate and ammonium contents were determined according to the national standard of China [35].

Soil polyphenol oxidase activity was estimated using the Polyphenol Oxidase Activity Assay Kit (code: D799508-0100; manufacturer: Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China) with micromethod. Soil FDA hydrolase activity was estimated using the Soil FDA Hydrolase Activity Assay Kit (code: D799552-0100; manufacturer: Sangon Biotech (Shanghai) Co., Ltd., China) with micromethod. Soil cellulase activity was measured using the Soil Cellulase Activity Assay Kit (code: D799514-0100; manufacturer: Sangon Biotech (Shanghai) Co., Ltd., China) with micromethod. Soil  $\beta$ -glucosidase activity was estimated using the Soil  $\beta$ -glucosidase Activity Assay Kit (code: D799510-0100; manufacturer: Sangon Biotech (Shanghai) Co., Ltd., China) with micromethod. Soil  $\beta$ -xylosidase activity was estimated using the Soil  $\beta$ -xylosidase Activity Assay Kit (code: D799556-0100; manufacturer: Sangon Biotech (Shanghai) Co., Ltd., China) with micromethod. Soil sucrase activity was estimated using the Soil Sucrase Activity Assay Kit (code: D799504-0100; manufacturer: Sangon Biotech (Shanghai) Co., Ltd., China) with micromethod. Soil protease activity was estimated using the Soil Protease Activity Assay Kit (code: D799540-0100; manufacturer: Sangon Biotech (Shanghai) Co., Ltd., China) with micromethod. Soil urease activity was estimated using the Soil Urease Activity Assay Kit (code: D799506-0100; manufacturer: Sangon Biotech (Shanghai) Co., Ltd., China) with micromethod.

#### 2.6. Determination of the Structure of Soil Bacterial Community

The SBCS was determined by Illumina PE250 at GENE DENOVO Co., Ltd., Guangzhou, Guangdong, China. The V3-V4 region of 16S rRNA genes of SBC was amplified using universal primers, i.e., 341F (5'-CCT AYG GGR BGC ASC AG-3')/806R (5'-GGA CTA CNN GGG TAT CTA AT-3') [36,37]. Other methods used to analyze SBCS were described in our previous studies [38].

#### 2.7. Statistical Analysis

Differences in the variables between different treatments were evaluated using one-way analysis of variance (ANOVA) with Duncan's test. Correlation patterns between soil physicochemical properties and enzyme activities, SBAD, and the decomposition coefficient were evaluated using correlation analysis and principal components analysis (PCA). The significance level was set at  $p \leq 0.05$ . The statistical analyses were performed using the IBM SPSS Statistics 26.0 (IBM, Inc., Armonk, NY, USA).

### 3. Results

#### 3.1. Differences in Decomposition Rate

After seven months of decomposition, the decomposition rate of the four AIPs and *P. laciniata* under the mixed nitrogen forms was significantly higher than that under the control ( $p < 0.05$ ; Table 1).

The decomposition rate of *C. canadensis* was significantly higher than that of *S. subulatum* ( $p < 0.05$ ; Table 2).

#### 3.2. Differences in Soil Physicochemical Properties and Enzyme Activities

Soil organic carbon under nitrate was significantly lower than that under the control ( $p < 0.05$ ; Table 1). Soil nitrate content under nitrate and urea was significantly higher than that under the control ( $p < 0.05$ ; Table 1). Soil ammonium content under three individual nitrogen forms was significantly lower than that under the control ( $p < 0.05$ ; Table 1). Soil  $\beta$ -xylosidase activity under D-AN with four forms was significantly lower than that under the control ( $p < 0.05$ ; Table 1). Soil sucrase activity under urea was significantly higher than that under the control ( $p < 0.05$ ; Table 1). There were no significant differences in other indices of soil physicochemical properties and enzyme activities under D-AN with four forms and the control ( $p > 0.05$ ; Table 1).

Soil moisture treated with *S. canadensis* and *S. subulatum* leaves was significantly lower than that treated with *P. laciniata* leaves ( $p < 0.05$ ; Table 2). Soil electrical conductivity treated with *S. canadensis* leaves was significantly lower than that treated with *B. pilosa* and *C. canadensis* leaves ( $p < 0.05$ ; Table 2). Soil polyphenol oxidase activity treated with *C. canadensis* and *S. subulatum* leaves was significantly higher than that treated with *P. laciniata* leaves ( $p < 0.05$ ; Table 2). Soil FDA hydrolase activity treated with *S. subulatum* leaves was significantly lower than that treated with the leaves of other plants ( $p < 0.05$ ; Table 2). Soil  $\beta$ -glucosidase activity treated with *S. subulatum* leaves was significantly lower than that treated with *B. pilosa* and *C. canadensis* leaves ( $p < 0.05$ ; Table 2). Soil sucrase activity treated with *C. canadensis*, *S. canadensis*, and *S. subulatum* leaves was significantly lower than that treated with *B. pilosa* leaves ( $p < 0.05$ ; Table 2). Soil sucrase activity treated with *S. canadensis* and *S. subulatum* leaves was also significantly lower than that treated with *P. laciniata* leaves ( $p < 0.05$ ; Table 2). There were no significant differences in other indices of soil physicochemical properties and enzyme activities treated with the leaves of the four AIPs and *P. laciniata* ( $p > 0.05$ ; Table 2).

#### 3.3. Differences in Soil Bacterial Alpha Diversity

The OTU's species index, Chao1's richness index, ACE's richness index, and Phylogenetic diversity index of the soil bacterial community under D-AN with four forms were significantly higher than those under the control ( $p < 0.05$ ; Table 1). There were no significant differences in the other indices of SBAD under D-AN with four forms and the control ( $p > 0.05$ ; Table 1).

The Phylogenetic diversity index of the soil bacterial community treated with *S. canadensis* leaves was significantly lower than that treated with *B. pilosa* leaves ( $p < 0.05$ ; Table 2). There were no significant differences in the other indices of SBAD treated with the leaves of the four AIPs and *P. laciniata* ( $p > 0.05$ ; Table 2).

#### 3.4. Correlation Patterns between Soil Physicochemical Properties and Enzyme Activities, SBAD, and Decomposition Coefficient

Soil electrical conductivity, soil nitrate content, soil FDA hydrolase activity, soil  $\beta$ -glucosidase activity, soil  $\beta$ -xylosidase activity, and soil urease activity were positively related to the decomposition coefficient ( $p < 0.05$ ; Table 3).

**Table 1.** The decomposition coefficient, soil physicochemical properties and enzyme activities, and SBAD under D-AN with four forms. Data (means and SE;  $n = 18$ ) with different letters in a transverse column indicate statistically significant differences ( $p < 0.05$ ). “ns” means no statistically significant difference ( $p > 0.05$ ). Abbreviations: CK, control; MixN, mixed nitrogen forms.

	CK	Nitrate	Ammonium	Urea	MixN
Decomposition coefficient	1.54 ± 0.36 b	2.12 ± 0.16 ab	2.7 ± 0.5 ab	3.01 ± 0.59 ab	3.69 ± 0.45 a
Soil pH	6.8 ± 0.00 ns	6.83 ± 0.02 ns	6.77 ± 0.02 ns	6.76 ± 0.04 ns	6.74 ± 0.06 ns
Soil moisture	0.38 ± 0.02 ns	0.41 ± 0.01 ns	0.42 ± 0.05 ns	0.43 ± 0.02 ns	0.44 ± 0.04 ns
Soil electrical conductivity	0.11 ± 0.02 ns	0.09 ± 0.01 ns	0.1 ± 0.02 ns	0.09 ± 0.01 ns	0.09 ± 0.01 ns
Soil organic carbon content	14.73 ± 1.09 a	12.86 ± 0.21 b	13.68 ± 0.47 ab	13.44 ± 0.42 ab	13.42 ± 0.38 ab
Soil microbial carbon content	67.81 ± 2.07 ns	69.16 ± 1.20 ns	69.3 ± 1.37 ns	64.5 ± 1.13 ns	65.63 ± 2.65 ns
Soil organic nitrogen content	4.8 ± 0.32 ns	4.49 ± 0.27 ns	4.34 ± 0.24 ns	4.71 ± 0.18 ns	4.05 ± 0.29 ns
Soil microbial nitrogen content	8.27 ± 2.78 ns	8.29 ± 0.49 ns	9.38 ± 0.45 ns	8.58 ± 0.62 ns	9.38 ± 0.65 ns
Soil nitrate content	11.72 ± 0.33 b	18.43 ± 1.55 a	14.63 ± 1.34 ab	18.8 ± 1.64 a	16.08 ± 1.26 ab
Soil ammonium content	0.87 ± 0.09 a	0.68 ± 0.04 b	0.68 ± 0.05 b	0.65 ± 0.02 b	0.74 ± 0.06 ab
Soil polyphenol oxidase activity	28.42 ± 4.01 ns	26.38 ± 2.08 ns	28.99 ± 2.00 ns	27.1 ± 1.73 ns	31.72 ± 2.09 ns
Soil FDA hydrolase activity	3588 ± 664.93 ns	3027.33 ± 27.00 ns	2738 ± 202.72 ns	3020.67 ± 207.84 ns	2822.67 ± 285.71 ns
Soil cellulase activity	34.67 ± 0.83 ns	38.78 ± 2.57 ns	36.3 ± 2.64 ns	36.45 ± 1.59 ns	38.31 ± 2.45 ns
Soil β-glucosidase activity	12.24 ± 0.50 ns	13.48 ± 0.97 ns	13.23 ± 0.53 ns	13.37 ± 0.91 ns	12.57 ± 0.46 ns
Soil β-xylosidase activity	5.67 ± 0.30 a	5.02 ± 0.13 b	5.06 ± 0.13 b	5.11 ± 0.11 b	5.15 ± 0.12 b
Soil sucrase activity	16.03 ± 4.13 b	25.41 ± 3.90 ab	16.99 ± 2.73 ab	29.82 ± 5.56 a	21.41 ± 2.87 ab
Soil protease activity	0.38 ± 0.01 ns	0.37 ± 0.02 ns	0.36 ± 0.02 ns	0.37 ± 0.03 ns	0.37 ± 0.01 ns
Soil urease activity	90.13 ± 2.23 ns	99.95 ± 5.86 ns	99.3 ± 4.63 ns	112.44 ± 7.23 ns	92.16 ± 11.48 ns
OTU's species index	3036.67 ± 323.39 b	3504.78 ± 136.46 a	3741.55 ± 104.60 a	3582.17 ± 131.50 a	3674 ± 72.82 a
Shannon's diversity index	8.95 ± 0.17 ns	9.02 ± 0.15 ns	9.14 ± 0.11 ns	9.25 ± 0.04 ns	9.23 ± 0.10 ns
Simpson's dominance index	0.99 ± 0.00 ns	0.99 ± 0.00 ns			
Pielou's evenness index	0.78 ± 0.01 ns	0.77 ± 0.01 ns	0.77 ± 0.01 ns	0.79 ± 0.00 ns	0.78 ± 0.01 ns
Chao1's richness index	3563.04 ± 246.47 b	4132.47 ± 138.46 a	4338.73 ± 121.98 a	4153.33 ± 170.35 a	4289.46 ± 75.54 a
ACE's richness index	3692.02 ± 246.08 b	4363.74 ± 147.94 a	4587.5 ± 131.23 a	4352.47 ± 191.36 a	4511.73 ± 84.26 a
Phylogenetic diversity index	368.45 ± 33.46 b	421.12 ± 13.84 a	446.85 ± 10.13 a	432.94 ± 9.49 a	435.39 ± 6.63 a

**Table 2.** The decomposition coefficient, soil physicochemical properties and enzyme activities, and SBAD treated with the leaves of the four AIPs and *P. laciniata*. Data (means and SE;  $n = 15$ ) with different letters in a transverse column indicate statistically significant differences ( $p < 0.05$ ). “ns” means no statistically significant difference ( $p > 0.05$ ). Abbreviations: PL, *Pterocypsela laciniata* (Houtt.) Shih; BP, *Bidens pilosa* L.; CC, *Conyza canadensis* (L.) Cronq.; SC, *Solidago canadensis* L.; SS, *Symphotrichum subulatum* (Michx.) G.L. Nesom.

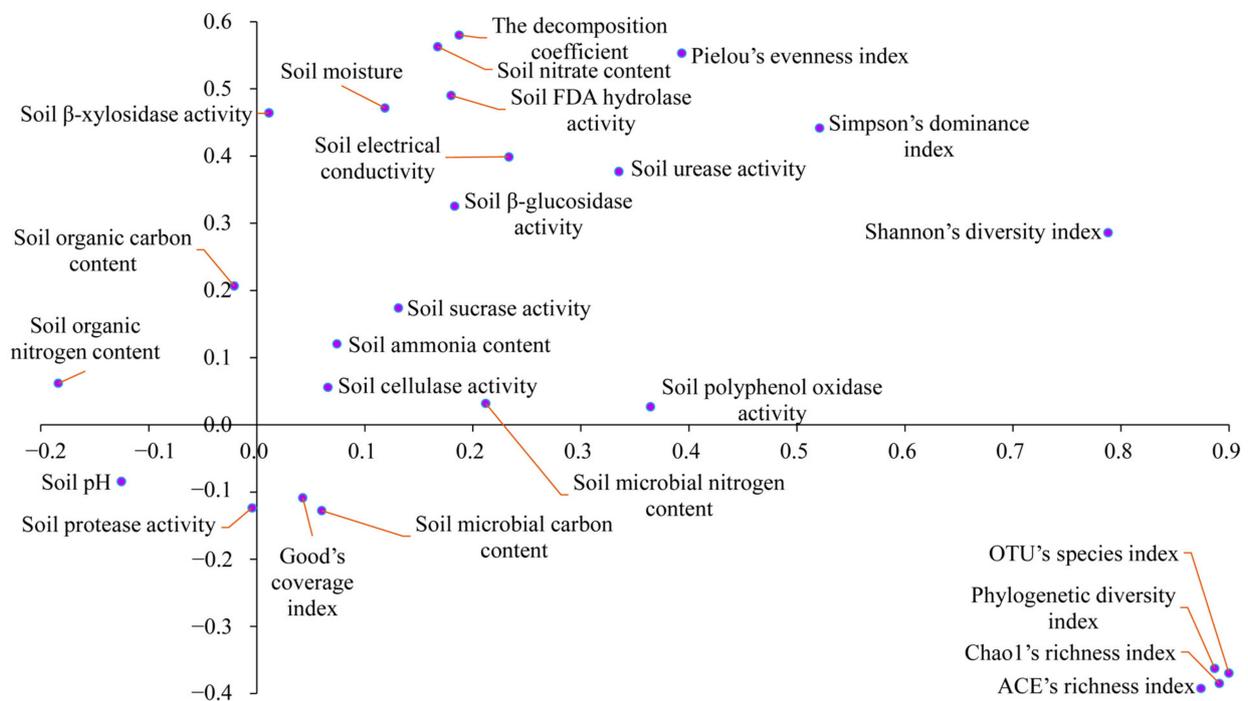
	PL	BP	CC	SC	SS
Decomposition coefficient	2.94 ± 0.67 ab	2.74 ± 0.48 ab	3.37 ± 0.57 a	2.37 ± 0.39 ab	1.65 ± 0.23 b
Soil pH	6.82 ± 0.03 ns	6.75 ± 0.03 ns	6.79 ± 0.03 ns	6.84 ± 0.01 ns	6.78 ± 0.04 ns
Soil moisture	0.47 ± 0.04 a	0.43 ± 0.02 ab	0.41 ± 0.03 ab	0.35 ± 0.02 b	0.36 ± 0.03 b
Soil electrical conductivity	0.08 ± 0.01 ab	0.1 ± 0.01 a	0.09 ± 0.00 a	0.05 ± 0.01 b	0.08 ± 0.01 ab
Soil organic carbon content	13.38 ± 0.46 ns	12.86 ± 0.20 ns	13.5 ± 0.50 ns	13.02 ± 0.53 ns	13.28 ± 0.40 ns
Soil microbial carbon content	68.95 ± 1.93 ns	66.51 ± 1.07 ns	67.7 ± 2.09 ns	65.3 ± 1.34 ns	64.9 ± 2.64 ns
Soil organic nitrogen content	4.33 ± 0.29 ns	4.4 ± 0.26 ns	4.33 ± 0.34 ns	4.81 ± 0.18 ns	4.32 ± 0.20 ns
Soil microbial nitrogen content	8.56 ± 0.96 ns	9.49 ± 0.68 ns	8.82 ± 0.74 ns	7.89 ± 0.57 ns	8.76 ± 0.55 ns
Soil nitrate content	18.96 ± 2.44 ns	15.8 ± 2.87 ns	15.52 ± 2.46 ns	14.26 ± 2.11 ns	12.12 ± 1.59 ns
Soil ammonium content	0.7 ± 0.07 ns	0.66 ± 0.05 ns	0.75 ± 0.06 ns	0.62 ± 0.03 ns	0.66 ± 0.02 ns
Soil polyphenol oxidase activity	24.96 ± 1.71 b	29.57 ± 2.56 ab	33.47 ± 0.77 a	29 ± 1.99 ab	31.36 ± 1.30 a
Soil FDA hydrolase activity	2839.2 ± 178.87 a	3168 ± 154.72 a	3171.2 ± 258.25 a	2888.8 ± 273.47 a	2074 ± 154.99 b
Soil cellulase activity	35.55 ± 1.24 ns	39.71 ± 2.31 ns	42.74 ± 3.75 ns	38.69 ± 4.06 ns	34.68 ± 0.56 ns
Soil β-glucosidase activity	13.21 ± 0.50 ab	13.73 ± 0.82 a	13.99 ± 0.66 a	13.01 ± 1.15 ab	11.08 ± 0.41 b
Soil β-xylosidase activity	5.24 ± 0.13 ns	4.99 ± 0.09 ns	5.2 ± 0.06 ns	4.99 ± 0.18 ns	5.11 ± 0.16 ns
Soil sucrase activity	25.54 ± 2.23 ab	33.7 ± 2.75 a	21.44 ± 2.85 bc	14.83 ± 4.17 c	13.66 ± 1.80 c
Soil protease activity	0.36 ± 0.01 ns	0.4 ± 0.03 ns	0.41 ± 0.02 ns	0.37 ± 0.03 ns	0.37 ± 0.02 ns
Soil urease activity	90.23 ± 12.43 ns	97.82 ± 6.17 ns	111.45 ± 4.42 ns	110.67 ± 9.74 ns	97.92 ± 8.08 ns
OTU's species index	3603.93 ± 114.99 ns	3847.13 ± 97.5 ns	3740.8 ± 53.99 ns	3594.33 ± 124.32 ns	3658.5 ± 129.07 ns
Shannon's diversity index	9.08 ± 0.14 ns	9.24 ± 0.13 ns	9.25 ± 0.06 ns	9.12 ± 0.08 ns	9.27 ± 0.05 ns
Simpson's dominance index	0.99 ± 0.00 ns	0.99 ± 0.00 ns	0.99 ± 0.00 ns	0.99 ± 0.00 ns	0.99 ± 0.00 ns
Pielou's evenness index	0.77 ± 0.01 ns	0.78 ± 0.01 ns	0.78 ± 0.01 ns	0.77 ± 0.01 ns	0.79 ± 0.00 ns
Chao1's richness index	4144.24 ± 128.64 ns	4477.88 ± 94.19 ns	4407.77 ± 66.63 ns	4232.75 ± 117.91 ns	4292.45 ± 162.42 ns
ACE's richness index	4342.18 ± 141.83 ns	4703.6 ± 104.27 ns	4671.17 ± 83.87 ns	4475.48 ± 127.43 ns	4546.44 ± 185.6 ns
Phylogenetic diversity index	426.74 ± 11.13 ab	457.37 ± 9.50 a	444.33 ± 4.62 ab	422.4 ± 11.78 b	438.97 ± 10.28 ab

**Table 3.** Correlations (*r*, Pearson’s coefficient) between soil physicochemical properties and enzyme activities, SBAD, and the decomposition coefficient (*k*) treated with the leaves of the four AIPs and *P. laciniata* under D-AN with four Forms.

		<i>k</i>			<i>k</i>			<i>k</i>
Soil pH	<i>r</i>	0.045	Soil ammonium content	<i>r</i>	0.004	Soil urease activity	<i>r</i>	0.291 *
	<i>p</i>	0.699		<i>p</i>	0.972		<i>p</i>	0.011
Soil moisture	<i>r</i>	0.175	Soil polyphenol oxidase activity	<i>r</i>	0.102	OTU’s species index	<i>r</i>	0.012
	<i>p</i>	0.133		<i>p</i>	0.385		<i>p</i>	0.919
Soil electrical conductivity	<i>r</i>	0.239 *	Soil FDA hydrolase activity	<i>r</i>	0.386 ***	Shannon’s diversity index	<i>r</i>	0.179
	<i>p</i>	<b>0.039</b>		<i>p</i>	<b>&lt;0.001</b>		<i>p</i>	0.125
Soil organic carbon content	<i>r</i>	0.088	Soil cellulase activity	<i>r</i>	0.034	Simpson’s dominance index	<i>r</i>	0.167
	<i>p</i>	0.452		<i>p</i>	0.771		<i>p</i>	0.153
Soil microbial carbon content	<i>r</i>	−0.128	Soil β-glucosidase activity	<i>r</i>	0.256 *	Pielou’s evenness index	<i>r</i>	0.195
	<i>p</i>	0.273		<i>p</i>	<b>0.026</b>		<i>p</i>	0.094
Soil organic nitrogen content	<i>r</i>	−0.016	Soil β-xylosidase activity	<i>r</i>	0.296 **	Chao1’s richness index	<i>r</i>	0.015
	<i>p</i>	0.892		<i>p</i>	<b>0.010</b>		<i>p</i>	0.899
Soil microbial nitrogen content	<i>r</i>	0.005	Soil sucrose activity	<i>r</i>	0.108	ACE’s richness index	<i>r</i>	0.002
	<i>p</i>	0.969		<i>p</i>	0.359		<i>p</i>	0.986
Soil nitrate content	<i>r</i>	<b>0.375 ***</b>	Soil protease activity	<i>r</i>	0.093	Phylogenetic diversity index	<i>r</i>	−0.025
	<i>p</i>	<b>&lt;0.001</b>		<i>p</i>	0.427		<i>p</i>	0.831

\*, \*\*, and \*\*\* indicate statistically significant differences at the 0.05, 0.01, and 0.001 probability levels, respectively. *p* values equal to or less than 0.05 are shown in bold.

Strong correlations were observed between the decomposition coefficient and soil moisture, soil electrical conductivity, soil nitrate content, soil FDA hydrolase activity, soil urease activity, and Pielou’s evenness index of the soil bacterial community (Figure 1).



**Figure 1.** The PCA of correlation patterns of the decomposition coefficient, soil physicochemical properties and enzyme activities, and soil bacterial alpha diversity treated with the leaves of the four AIPs and *P. laciniata* under D-AN with four forms. The X-axis and the Y-axis account for 18.35% and 11.69% of the total variation, respectively.

### 3.5. Differences in the Structure of Soil Bacterial Community

The average value of Good's coverage index of the soil bacterial community across all samples was ~0.9822. Soil bacterial beta diversity under D-AN with four forms and that treated with the leaves of the four AIPs and *P. laciniata* showed noticeable differences based on weighted UniFrac distances (Figures S1 and S2).

At the order level, the dominant role of predominant soil bacterial biomarkers of Propionibacteriales and Cytophagales was apparently reduced, but the dominant role of predominant soil bacterial biomarkers of Saccharimonadales, Flavobacteriales, and Sphingomonadales was apparently enhanced under D-AN with four forms compared to that under the control (Figure S3).

At the order level, the dominant role of predominant soil bacterial biomarkers of Burkholderiales was apparently reduced, but the dominant role of predominant soil bacterial biomarkers of Rhizobiales and Propionibacteriales was apparently enhanced when treated with the leaves of the four AIPs and *P. laciniata* under nitrate compared to that treated with nitrate (Figure S4). At the order level, the dominant role of predominant soil bacterial biomarkers of Flavobacteriales, Cytophagales, Chitinophagales, and Sphingomonadales was apparently reduced, but the dominant role of predominant soil bacterial biomarkers of Rhizobiales and Chloroplast was apparently enhanced when treated with the leaves of the four AIPs under nitrate compared to that treated with *P. laciniata* leaves under nitrate (Figure S4).

At the order level, the dominant role of predominant soil bacterial biomarkers of Burkholderiales was apparently reduced, but the dominant role of predominant soil bacterial biomarkers of Rhizobiales was apparently enhanced when treated with the leaves of the four AIPs and *P. laciniata* under ammonium compared to that treated with ammonium (Figure S5). At the order level, the dominant role of predominant soil bacterial biomarkers of Xanthomonadales and Cytophagales was apparently reduced, but the dominant role of predominant soil bacterial biomarkers of Chloroplast was apparently enhanced when treated with the leaves of the four AIPs under ammonium compared to that treated with *P. laciniata* leaves under ammonium (Figure S5).

At the order level, the dominant role of predominant soil bacterial biomarkers of Rhizobiales was apparently enhanced when treated with the leaves of the four AIPs and *P. laciniata* under urea compared to that treated with urea (Figure S6). At the order level, the dominant role of predominant soil bacterial biomarkers of Flavobacteriales, Cytophagales, and Chitinophagales was apparently reduced, but the dominant role of predominant soil bacterial biomarkers of Chloroplast was apparently enhanced when treated with the leaves of the four AIPs under urea compared to that treated with *P. laciniata* leaves under urea (Figure S6).

At the order level, the dominant role of predominant soil bacterial biomarkers of Rhizobiales was apparently enhanced when treated with the leaves of the four AIPs and *P. laciniata* under the mixed nitrogen forms compared to that treated with the mixed nitrogen forms (Figure S7). At the order level, the dominant role of predominant soil bacterial biomarkers of Flavobacteriales, Cytophagales, and Sphingomonadales was apparently reduced, but the dominant role of predominant soil bacterial biomarkers of Rhizobiales and Chloroplast was apparently enhanced when treated with the leaves of the four AIPs under the mixed nitrogen forms compared to that treated with *P. laciniata* leaves under the mixed nitrogen forms (Figure S7).

### 3.6. Differences in the Number of Functional Gene Pathways of Soil Bacterial Community Involved in the Decomposition Process under D-AN with Four Forms

The number of pathways of several functional genes of the soil bacterial community involved in the decomposition process significantly increased under D-AN with four forms compared to that under the control, including L-arginine degradation II (AST pathway), Catechol degradation to  $\beta$ -keto adipate, Fucose degradation, D-galactarate degradation I, D-glucarate degradation I, Superpathway of D-glucarate and D-galactarate degrada-

tion, Superpathway of  $\beta$ -D-glucuronide and D-glucuronate degradation, Methylgallate degradation, Protocatechuate degradation II (ortho-cleavage pathway), Benzoyl-coa degradation I (aerobic), Superpathway of taurine degradation, Toluene degradation IV (aerobic) (via catechol), Superpathway of aerobic toluene degradation, Superpathway of salicylate degradation, 2-aminophenol degradation, Galactose degradation I (Leloir pathway), L-tryptophan degradation XII (Geobacillus), 4-coumarate degradation (anaerobic), Nicotinate degradation I, 3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation, Methylphosphonate degradation I, and L-valine degradation I (Table S1). However, D-AN with four forms significantly decreased the number of pathways of S-methyl-5-thio- $\alpha$ -D-ribose 1-phosphate degradation compared to that under the control (Table S1).

The number of pathways of Benzoyl-coa degradation II (anaerobic) also significantly increased under three individual nitrogen forms compared to that under the control (Table S1). The number of pathways of Adenosine nucleotides degradation IV also significantly increased under ammonium and the mixed nitrogen forms compared to that under the control (Table S1).

The number of pathways of several functional genes of the soil bacterial community involved in the decomposition process also significantly increased under ammonium, urea, and the mixed nitrogen forms compared to that under the control, including 4-hydroxyphenylacetate degradation, Superpathway of hexuronide and hexuronate degradation, D-galacturonate degradation I, Glycogen degradation I (bacterial), Syringate degradation, Purine nucleotides degradation II (aerobic), Guanosine nucleotides degradation III, Superpathway of sulfolactate degradation, Starch degradation V, Superpathway of glucose and xylose degradation, Androstenedione degradation, D-fructuronate degradation, Aromatic biogenic amine degradation (bacteria), and Adenosine nucleotides degradation II (Table S1).

The number of pathways of several functional genes of the soil bacterial community involved in the decomposition process also significantly increased under nitrate, urea, and the mixed nitrogen forms compared to that under the control, including Glucose degradation (oxidative), Gallate degradation II, Protocatechuate degradation I (meta-cleavage pathway), Catechol degradation III (ortho-cleavage pathway), Aromatic compounds degradation via  $\beta$ -keto adipate, Superpathway of vanillin and vanillate degradation (Table S1).

The number of pathways of several functional genes of the soil bacterial community involved in the decomposition process also significantly increased under urea and the mixed nitrogen forms compared to that under the control, including 3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation to 2-oxopent-4-enoate, Superpathway of L-arginine and L-ornithine degradation, Purine nucleobases degradation I (anaerobic), 3-phenylpropanoate degradation, Allantoin degradation to glyoxylate III, Superpathway of phenylethylamine degradation, Cinnamate and 3-hydroxycinnamate degradation to 2-oxopent-4-enoate, and L-rhamnose degradation I (Table S1).

The number of pathways of several functional genes of the soil bacterial community involved in the decomposition process also significantly increased under urea compared to that under the control, including Superpathway of L-arginine, putrescine, and 4-aminobutanoate degradation, Superpathway of N-acetylglucosamine, N-acetylmannosamine and N-acetylneuraminic acid degradation, Superpathway of methylglyoxal degradation, Superpathway of N-acetylneuraminic acid degradation, Glycine betaine degradation I, Toluene degradation III (aerobic) (via p-cresol), Urate biosynthesis/inosine 5'-phosphate degradation, Glycogen degradation II (eukaryotic), Chondroitin sulfate degradation I (bacterial), Chitin derivatives degradation, L-arabinose degradation IV, Heparin degradation, and Purine ribonucleosides degradation (Table S1).

The number of pathways of several functional genes of the soil bacterial community involved in the decomposition process also significantly increased under the mixed nitrogen forms compared to that under the control, including Myo-inositol degradation I, Superpathway of pyrimidine ribonucleosides degradation, and Myo-, chiro- and scillo-inositol degradation (Table S1).

### 3.7. Differences in the Number of Functional Gene Pathways of Soil Bacterial Community Involved in the Decomposition Process Treated with the Leaves of the Four AIPs and *P. laciniata*

The number of pathways of several functional genes of the soil bacterial community involved in the decomposition process significantly decreased when treated with the leaves of the four AIPs compared to that treated with *P. laciniata* leaves, including L-histidine degradation I, L-leucine degradation I, 2-nitrobenzoate degradation I, L-tryptophan degradation to 2-amino-3-carboxymuconate semialdehyde, 2-amino-3-carboxymuconate semialdehyde degradation to 2-oxopentenoate, L-tryptophan degradation IX, L-1,2-propanediol degradation (Table S2).

*Bidens pilosa* leaves significantly increased the number of pathways of Creatinine degradation I, Sucrose degradation II (sucrose synthase), and Aromatic biogenic amine degradation (bacteria), but significantly decreased the number of pathways of L-arginine degradation II (AST pathway), Gallate degradation I, Superpathway of glycerol degradation to 1,3-propanediol, 3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation to 2-oxopent-4-enoate, Superpathway of vanillin and vanillate degradation, Cinnamate and 3-hydroxycinnamate degradation to 2-oxopent-4-enoate, Androstenedione degradation, Sitosterol degradation to androstenedione, Vanillin and vanillate degradation I, Vanillin and vanillate degradation II, and 3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation compared to that treated with *P. laciniata* leaves (Table S2).

*Conyza canadensis* leaves significantly increased the number of pathways of 4-hydroxyphenylacetate degradation, Creatinine degradation I, 3-phenylpropanoate degradation, Myo-inositol degradation I, Glycine betaine degradation I, Toluene degradation IV (aerobic) (via catechol), Superpathway of aerobic toluene degradation, Allantoin degradation to glyoxylate III, Myo-, chiro- and scillo-inositol degradation, and Methylphosphonate degradation I, but significantly decreased the number of pathways of Sitosterol degradation to androstenedione compared to that treated with *P. laciniata* leaves (Table S2).

*Solidago canadensis* leaves significantly increased the number of pathways of Acetylene degradation, 3-phenylpropanoate degradation, Myo-inositol degradation I, Toluene degradation IV (aerobic) (via catechol), Superpathway of aerobic toluene degradation, P-cymene degradation, Allantoin degradation to glyoxylate III, 4-hydroxyacetophenone degradation, Nicotinate degradation I, Myo-, chiro- and scillo-inositol degradation, Aromatic biogenic amine degradation (bacteria), and Methylphosphonate degradation I, but significantly decreased the number of pathways of L-arginine degradation II (AST pathway), 3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation to 2-oxopent-4-enoate, L-histidine degradation II, Catechol degradation to 2-oxopent-4-enoate II, Catechol degradation II (meta-cleavage pathway), L-tryptophan degradation XII (Geobacillus), 4-deoxy-L-threo-hex-4-enopyranuronate degradation, Guanosine nucleotides degradation III, Cinnamate and 3-hydroxycinnamate degradation to 2-oxopent-4-enoate, Starch degradation III, Sitosterol degradation to androstenedione, Heparin degradation, 3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation, Phenylacetate degradation I (aerobic), and L-tyrosine degradation I compared to that treated with *P. laciniata* leaves (Table S2).

*Symphotrichum subulatum* leaves significantly increased the number of pathways of Superpathway of hexitol degradation (bacteria), 3-phenylpropanoate degradation, Chondroitin sulfate degradation I (bacterial), Aromatic biogenic amine degradation (bacteria), Mannan degradation, but significantly decreased the number of pathways of L-arginine degradation II (AST pathway), Creatinine degradation I, Gallate degradation I, Superpathway of glycerol degradation to 1,3-propanediol, 3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation to 2-oxopent-4-enoate, Protocatechuate degradation II (ortho-cleavage pathway), L-histidine degradation II, Toluene degradation I (aerobic) (via o-cresol), Toluene degradation III (aerobic) (via p-cresol), Toluene degradation II (aerobic) (via 4-methylcatechol), Catechol degradation I (meta-cleavage pathway), Catechol degradation to 2-oxopent-4-enoate II, Catechol degradation II (meta-cleavage pathway), Urate biosynthesis/inosine 5'-phosphate degradation, Superpathway of phenylethylamine degradation, 2-aminophenol degradation, Superpathway of vanillin and vanillate degrada-

tion, L-tryptophan degradation XII (Geobacillus), Guanosine nucleotides degradation III, Superpathway of sulfolactate degradation, Cinnamate and 3-hydroxycinnamate degradation to 2-oxopent-4-enoate, Starch degradation III, Androstenedione degradation, Sitosterol degradation to androstenedione, Vanillin and vanillate degradation I, Vanillin and vanillate degradation II, 3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation, Phenylacetate degradation I (aerobic), Adenosine nucleotides degradation II, and L-tyrosine degradation I compared to that treated with *P. laciniata* leaves (Table S2).

#### 4. Discussion

In this study, the mixed nitrogen forms significantly increased the decomposition rate of the four AIPs and *P. laciniata*. This may be due to a decrease in the limitation of nitrogen utilization for the metabolic activity and diversity of the soil bacterial community involved in the decomposition process [39,40]. This phenomenon can also be attributed to the fact that D-AN may trigger a priming effect on the metabolic activity and diversity of some components of the soil microbial community involved in the decomposition process, especially under a low level of nutrient (especially nitrogen) availability [39,41]. More importantly, the results of this study also showed that D-AN with four forms all significantly increased the species number, richness, and phylogenetic diversity of the soil bacterial community, as well as the number of most of the functional gene pathways of the soil bacterial communities involved in the decomposition process.

Among the four forms of D-AN, the positive effects of the mixed nitrogen forms on the decomposition rate of the four AIPs and *P. laciniata*, and the number of several functional gene pathways of the soil bacterial communities involved in the decomposition process were obviously greater than those of individual nitrogen forms in this study. This finding may be due to (1) the occurrence of numerous functional groups within the soil microbial community, each specializing in the utilization of an individual nitrogen form, so that nitrogen demand of only one functional group is adequately met when only individual nitrogen form is fertilized, but all demands are adequately met when numerous nitrogen forms are fertilized, resulting in the maximization of benefits for the decomposition rate; (2) the possibility of a disturbance in the balance between inorganic and organic nitrogen for the metabolic activity and diversity of the soil bacterial community involved in the decomposition process only when an individual nitrogen form is fertilized, resulting in the attenuation of benefits to the decomposition rate when an individual nitrogen form is fertilized than when the mixed nitrogen forms are fertilized [42,43]; or (3) the possibility that the beneficial effects of organic nitrogen on the metabolic activity and diversity of the soil bacterial community may be greater than those of inorganic nitrogen since the soil bacterial community is more likely to utilize organic nitrogen than inorganic nitrogen [40,43,44].

The increased nitrate content and decreased ammonium content in soils under D-AN with four forms in this study may be because inorganic nitrogen can promote the nitrification process, and ammonium can be rapidly converted to nitrate [45–47].

The decreased soil organic carbon content under ammonium and the declined soil  $\beta$ -xylosidase activity under D-AN with four forms in this study may be attributed to the mitigating effects of D-AN with four forms, especially ammonium, on the decomposition efficiency of lignin [48–50]. Thus, with increasing D-AN (especially an increase in the ratio of ammonium in D-AN), more carbon (especially organic carbon) may be released from the soil system into the atmosphere, and thus D-AN may contribute to some extent to changing the role of the soil system as a carbon reservoir to that of a carbon source based on the results of this study.

In this study, D-AN with four forms visibly up- or down-regulated the dominant role of predominant soil bacterial biomarkers and the number of several functional gene pathways of the soil bacterial communities involved in the decomposition process. This finding may be attributed to the differences in the nitrogen-phototrophic ability of the predominant soil bacterial biomarkers, i.e., D-AN with four forms may exert different selection pressures on

different taxa of the predominant soil bacterial biomarkers, resulting in an enhancement in nitrogen-phototrophic taxa and a reduction in nitrogen-sensitive taxa.

The decomposition rates of the four AIPs and those of *P. laciniata* were not significantly different in this study. Thus, the four AIPs may not decompose more easily than those of *P. laciniata*. This phenomenon is not consistent with the current results, i.e., invasive plants decompose more easily than native plants [18,51–53] or invasive plants decompose more slowly than native plants [54–56]. This finding in this study may be due to the similar proportion of soluble (easily decomposed) and recalcitrant (difficult to decompose) components in the leaves of the four AIPs and those of *P. laciniata*, i.e., the quality of the leaves of the four AIPs and those of *P. laciniata* was similar because the four AIPs and *P. laciniata* are Asteraceae species and these species have similar life cycles, life styles, and growing environments, and especially *P. laciniata* can often coexist with one or more of the four AIPs. Due to the similar decomposition rate of the leaves of the four AIPs and those of *P. laciniata*, the decomposition process of the four AIPs did not significantly affect soil physicochemical properties (including soil carbon and nitrogen contents), most of the soil enzyme activities, and SBAD compared to that of *P. laciniata* in this study.

The leaves of *C. canadensis* were more easily decomposed than those of *S. subulatum* in this study. This finding may be due to the higher proportion of soluble components and the lower proportion of recalcitrant components in the leaves of *C. canadensis* compared to those of *S. subulatum*. Thus, the nutrient cycling rate during the decomposition process of *C. canadensis* may be apparently higher than that of *S. subulatum*. Accordingly, the decomposition process may play a more important role in the successful invasion of *C. canadensis* than that of *S. subulatum*.

Invasive plants can alter soil enzyme activity [57–60] by releasing nutrients, such as carbon- and nitrogen-containing substances, during the decomposition process. Surprisingly, the decomposition process of *S. subulatum* significantly increased soil polyphenol oxidase activity, but significantly decreased soil FDA hydrolase and sucrase activities compared to that of *P. laciniata* in this study. In addition, the decomposition process of *S. canadensis* significantly decreased soil sucrase activity compared to that of *P. laciniata* in this study. Thus, the effects of invasive plants on soil enzyme activities may be species specific.

The decomposition process of the four AIPs did not significantly affect SBAD but caused remarkable changes in the relative abundance of several taxa of the soil bacterial community and soil bacterial beta diversity, and triggered obvious up- or down-regulation in the dominant role of predominant soil bacterial biomarkers and in the number of several functional gene pathways of the soil bacterial communities involved in the decomposition process in this study. Hence, the decomposition process of the four AIPs may cause the appearance of numerous dominant biomarkers of the soil bacterial community. This phenomenon may be because there are still some differences in the quality of the leaves of the four AIPs and those of *P. laciniata*, resulting in a certain degree of species differentiation of the soil bacterial community. In particular, the altered relative abundance of several taxa of the soil bacterial community and up- or down-regulation in the dominant role of predominant soil bacterial biomarkers may be due to the selective facilitation and the selective inhibition activated by the altered type, amount, and complexity of nutrients, such as carbon- and nitrogen-containing substances and other recalcitrant components, in soils during the decomposition process. Therefore, the decomposition process of the four AIPs largely altered SBCS rather than SBAD. Previous studies have also confirmed this phenomenon [61,62].

In general, the decomposition rate of plant species is largely influenced by soil physicochemical properties and enzyme activities, as well as SBCS [12,24,42,63]. In this study, the decomposition rate of the four AIPs and *P. laciniata* may be mainly influenced by soil electrical conductivity, soil nitrate content, soil FDA hydrolase activity, and soil urease activity rather than SBCS, according to the results of the correlation analysis and PCA. This phenomenon may be possible because soil physicochemical properties and enzyme

activities may influence the release process of substances contained in the leaves during the decomposition process [12,42,53,64].

## 5. Conclusions

In summary, the mixed nitrogen forms significantly increased the decomposition rate of the four AIPs and *P. laciniata* compared to the control. Among the four forms of D-AN, the positive effects of the mixed nitrogen forms on the decomposition rate of the four AIPs and *P. laciniata* were obviously greater than those of individual nitrogen forms. In particular, D-AN with four forms significantly increased nitrate content but decreased ammonium content and  $\beta$ -xylosidase activity in soils. Ammonium significantly decreased soil organic carbon content. In addition, D-AN with four forms visibly up- or down-regulated the dominant role of predominant soil bacterial biomarkers. Ammonium significantly increased the species number, richness, and phylogenetic diversity of the soil bacterial community, as well as the number of most of the functional gene pathways of the soil bacterial communities involved in the decomposition process. The decomposition rate of the four AIPs was similar to that of *P. laciniata*. The leaves of *C. canadensis* decomposed more easily than those of *S. subulatum*. The effects of invasive plants on soil enzyme activities may be species specific. The decomposition process of the four AIPs caused remarkable changes in the relative abundance of several taxa of the soil bacterial community and soil bacterial beta diversity, and induced apparent up- or down-regulation in the dominant role of predominant soil bacterial biomarkers as well as in the number of several functional gene pathways of the soil bacterial communities involved in the decomposition process. The decomposition rate of the four AIPs and *P. laciniata* may be mainly influenced by electrical conductivity, nitrate content, FDA hydrolase activity, and urease activity in soils rather than SBCS.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nitrogen5010014/s1>, Table S1: Differences in the number of functional gene pathways of soil bacterial communities involved in the decomposition process under nitrogen with four forms. Data (means and SE;  $n = 18$ ) with different letters in a transverse column indicate statistically significant differences ( $p < 0.05$ ). The number of functional gene pathways of soil bacterial communities of most samples that lower than one was not shown in this table. Abbreviations: CK, control; MixN, the mixed nitrogen forms; Table S2: Differences in the number of functional gene pathways of soil bacterial communities involved in the decomposition process treated with the leaves of the four invasive plants and *P. laciniata*. Data (means and SE;  $n = 15$ ) with different letters in a transverse column indicate statistically significant differences ( $p < 0.05$ ). The number of functional gene pathways of soil bacterial communities of most samples that lower than one was not shown in this table. Abbreviations: PL, *Pterocypsela laciniata* (Houtt.) Shih; BP, *Bidens pilosa* L.; CC, *Conyza canadensis* (L.) Cronq.; SC, *Solidago canadensis* L.; SS, *Symphytotrichum subulatum* (Michx.) G.L. Nesom; Figure S1: PCoA of beta diversity estimates of soil bacterial communities treated with the leaves of the four invasive plants and *P. laciniata* under nitrogen with four forms based on weighted UniFrac distance. Abbreviations: PL, *Pterocypsela laciniata* (Houtt.) Shih; BP, *Bidens pilosa* L.; CC, *Conyza canadensis* (L.) Cronq.; SC, *Solidago canadensis* L.; SS, *Symphytotrichum subulatum* (Michx.) G.L. Nesom; CK, control; MixN, the mixed nitrogen forms; Figure S2: NMDS of beta diversity estimates of soil bacterial communities treated with the leaves of the four invasive plants and *P. laciniata* under nitrogen with four forms based on weighted UniFrac distance. Abbreviations have the same meanings as defined in Figure S1; Figure S3: Bubble chart of soil bacterial biomarkers at the order level under nitrogen with four forms. Abbreviations have the same meanings as defined in Figure S1; Figure S4: Bubble chart of soil bacterial biomarkers at the order level treated with the leaves of the four invasive plants and *P. laciniata* under nitrate. Abbreviations have the same meanings as defined in Figure S1; Figure S5: Bubble chart of soil bacterial biomarkers at the order level treated with the leaves of the four invasive plants and *P. laciniata* under ammonium. Abbreviations have the same meanings as defined in Figure S1; Figure S6: Bubble chart of soil bacterial biomarkers at the order level treated with the leaves of the four invasive plants and *P. laciniata* under urea. Abbreviations have the same meanings as defined in Figure S1; Figure S7: Bubble chart of soil bacterial biomarkers at the order level treated with the leaves of the four invasive plants and *P. laciniata* under the mixed nitrogen forms. Abbreviations have the same meanings as defined in Figure S1.

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