

## Article

# Effects of Canada Goldenrod Invasion on Soil Extracellular Enzyme Activities and Ecoenzymatic Stoichiometry

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**Abstract:** The rapid expansion of Canada goldenrod (*Solidago canadensis* L.) in China has drawn considerable attention as it may not only decrease vegetation diversity but also alter soil nutrient cycling in the affected ecosystems. Soil extracellular enzymes mediate nutrient cycling by catalyzing the organic matter decomposition; however, the mechanisms by which alien plant invasion may affect soil extracellular enzymes remain unclear. The objective of this study was to investigate the responses of soil extracellular enzyme activities and ecoenzymatic stoichiometry to *S. canadensis* invasion. Several extracellular enzymatic activities related to carbon, nitrogen, and phosphorus cycling were measured using a fluorometric method. Ecoenzymatic stoichiometry was used as a proxy of soil microbial metabolic limitations. *S. canadensis* invasion appeared to be associated with decreased activities of enzymes and with substantial conversions of microbial metabolic carbon and nitrogen limitations. The changes in the activities of extracellular enzymes and the limitations of microbial metabolism were correlated with the alterations in the nutrient availability and resource stoichiometry in the soil. These findings reveal that the alterations in soil available nutrients associated with *S. canadensis* invasion may regulate extracellular enzymatic activities and cause microbial metabolic limitations, suggesting that *S. canadensis* invasion considerably affects biogeochemical cycling processes.

**Keywords:** alien plant invasion; soil microbial metabolic limitation; soil available nutrient availability; *Solidago canadensis* L.; *Phragmites australis*



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## 1. Introduction

Biological invasions are one of the major threats to functioning, economical use, biodiversity, and services of the global ecosystem [1–4]. Alien invasive plants alter the structure and composition of vegetation communities in the invaded ecosystem due to rapid growth, high reproductive, and spreading capacity [5,6]. There is no doubt the shifts in the vegetation communities can further impact numerous ecosystem processes and functions, thereby cause irreversible effects on the invaded ecosystems [7,8]. Potential threats of alien invasive plants have been studied in depth, and various possible invasion hypotheses have been suggested [1,6]. However, some of these hypotheses are either overlapped or imprecise, mostly due to the overwhelming diversity regarding alien invasive plant species and types of invaded ecosystems [9]. Hence, understanding the mechanistic framework by which the

alien invasive plants outcompete native plants is crucial for mitigating invader effects on ecosystems and for maintaining natural biodiversity and ecosystem functionality [9].

Soil nutrient availability is considered a potential major abiotic factor influencing the success of alien invasive plants. Compared with native plants, alien invasive plants typically show higher nutrient use efficiency and more flexible nutrient use strategies [6]. In nutrient-limited environments, alien invasive plants can outcompete native plants either due to greater assimilation of carbon (C) and/or other nutrients or due to switching their nutrient use strategy to a “resource conservative strategy” that lowers their nutrient requirements to sustain high growth rates [10]. Moreover, alien invasive plants can change soil available nutrient content by releasing particular substrate compounds, which elicits alterations in soil microbial communities and thereby facilitates higher growth rates in the invasive plant species [7]. Many studies reported that invasive plants typically produce higher quantity and quality of leaf litter and root exudates in terms of higher nitrogen (N) content, lower ratio of C to N, and lignin content, which leads to enhanced availability of N and/or phosphorus (P), and to an imbalance in nutrient stoichiometry in soils of invaded habitats [9,11–14]. In addition, alterations in substrate availability can also drive changes in soil microbial communities. Thus, alien invasive plants can increase substrate decomposition rates by modifying soil microbial community functions, which accelerates nutrient cycling and the subsequent release of nutrients into the soil, thereby causing a positive, self-reinforcing feedback mechanism of invasion [8,12,15]. Nevertheless, there is still a lack of a mechanistic framework to help understand how changes in soil available nutrients induced by invasive plants accelerate successful invasion.

Soil nutrient availability and nutrient turnover are mainly regulated by soil microbes through extracellular enzymes that help decompose soil organic matter (SOM) [5,16]. Numerous enzymes required for C, N, and P acquisition from soil are highly sensitive to changes in soil nutrients, therefore, their activities have been considered as an index of soil nutrient availability and stability [17]. Sinsabaugh et al. [18,19] developed a new approach to assess the energy and nutrient limitations in soil microbial metabolism using soil extracellular coenzymatic stoichiometry based on the ecological stoichiometry theory and metabolic theory of ecology [20]. Soil extracellular coenzymatic stoichiometry can help link soil nutrient availability with soil microbial nutrient acquisition strategies, which are affected by soil microbial metabolism demand, as soil microbes obtain and/or compete with plants for soil available nutrients during SOM decomposition [19,21,22]. Hence, it is necessary to identify how nutrient status and metabolic limitations of soil microbes vary during the succession of native and invasive vegetation in order to understand soil microbial responses to alien plant invasion processes and their effects on soil nutrient cycles.

Canada goldenrod (*Solidago canadensis* L.) has become one of the most rapidly expanding alien invasive plants in China after its introduction from North America as a horticultural plant in 1935 [23,24]. This species has since colonized large areas of disturbed and undisturbed land in Southeastern China, including original habitats of common reed (*Phragmites australis* (Cav.) Trin. ex Steud) [25,26]. The objective of the present study was to investigate the patterns of soil extracellular enzymes and coenzymatic stoichiometry to assess soil microbial metabolic limitations and to identify drivers of such alteration processes following invasion by *S. canadensis*. To test this, four isolated *P. australis* original transect lines were selected based on similar environmental conditions in a shoreside area. In each of the four isolated *P. australis* transect lines, three study sites with different *S. canadensis* invasive gradient were established to measure six extracellular enzymatic activities related to C, N, and P cycling and coenzymatic stoichiometry in the soil. Compared to native plants, *S. canadensis* produces higher litter input and root exudates (e.g., allelopathy exudates) into the soil [27–29]. Here, we tested two hypotheses: (1) *S. canadensis* invasion will increase activities of soil extracellular enzymes; and (2) *S. canadensis* invasion will affect microbial nutrient status and metabolic limitations, which should be reflected in soil extracellular coenzymatic stoichiometry. The response patterns of soil extracellular

enzymatic activities and microbial metabolic limitations to *S. canadensis* invasion revealed in the present study will improve the understanding of the changes in soil nutrient cycling during plant invasion processes.

## 2. Materials and Methods

### 2.1. Study Site Description

The study area was in an artificial urban green space located at a shoreside of a tributary of the Yangtze river (32°14' N, 119°29' E) near Zhenjiang City, China. In 2018, the annual air temperature and natural precipitation were 17.1 °C and 1272.1 mm, respectively [30]. Plant diversity in this artificial urban green space was low and *P. australis* was the originally dominant landscape vegetation to ornament and divide different functional areas. However, the artificial urban green space was recently invaded by *S. canadensis*, which spread from east to west. The invasion formed a mosaic pattern of *S. canadensis* and *P. australis* transect lines. The overall coverage of *P. australis* in the study area was >50%, and that of *S. canadensis* was approximately 35%.

Within the study area, four isolated transect lines were selected based on similar environmental conditions. For each of the four transect lines, three study sites were divided according to the different dominant plant communities in terms of *S. canadensis* invasive gradient in the *P. australis* original habitats. From east to west, these sites included (1) an *S. canadensis*-dominated site (SD; coverage:  $96.83 \pm 0.49\%$ ), (2) a site co-dominated (CD) by *S. canadensis* (coverage:  $45.88 \pm 14.22\%$ ) and *P. australis* (coverage:  $18.63 \pm 5.33\%$ ), and (3) a site dominated by *P. australis* (PD; coverage:  $77.60 \pm 3.37\%$ ) which showed no or only slight signs of invasion. At each study site, one experimental plot (1 × 1 m) with over 80% coverage by *S. canadensis*, *P. australis*, or both was established to account for vegetation diversity. The interval between two neighboring plots in each transect line had a minimum distance of 8 m. Hence, a total number of 12 experimental plots (3 three different dominant plant community study sites × 4 replication) were established.

### 2.2. Soil Sample Collection and Preparation

Soil samples (top soil, 0–15 cm depth) were collected from ten points along an S-shaped pattern in each experimental plot using a soil corer (2.4 cm diameter) in November 2018. Soil samples from each plot were mixed thoroughly to obtain one composite soil sample, and a total number of 12 composite soil samples were obtained. All composite soil samples were passed through a sieve (2 mm) to remove visible plant debris and stones and to homogenize before subdividing the samples for analyses. Each composite sample was divided into two portions, one of which was stored at 4 °C until analyzed for microbial biomass and extracellular enzymatic activity (EEA), and the second portion was air-dried for soil physicochemical property analyses. Soil physicochemical property and soil microbial biomass analyses were performed within two weeks of sample collection, while EEA analysis was done within 48 h after sampling.

### 2.3. Measurement of Soil Physicochemical and Microbial Biomass Properties

Soil moisture (SM) was measured as mass loss after oven-drying at 105 °C for 72 h. Soil pH was measured in soil suspensions of air-dried soil in deionized water at a ratio of 1:5 (weight to volume). Soil cation exchange capacity (CEC) was assessed following the ammonium acetate (pH 7.0) method according to Brown [31]. Soil organic C (SOC) and SOM (SOM =  $1.724 \times \text{SOC}$ ) content were assessed using the dichromate oxidation method reported by Cui et al. [32]. Soil dissolved organic C (DOC) content was measured according to Li et al. [33]. Soil total C (STC) content and N (STN) content were quantified using an elemental analyzer (vario MACRO; Elementar Analysensysteme GmbH, Langensfeld, Germany). Soil inorganic N (SIN) content was recorded as the sum of soil NO<sub>3</sub>-N and NH<sub>4</sub>-N contents which were measured following the colorimetric methods reported by Miranda et al. [34] and Mulvaney [35], respectively. Soil total P (STP) and available P (SAP) contents were measured following the molybdate colorimetric method from Murphy and

Riley [36] and Olsen et al. [37]. Soil microbial biomass C (MBC), N (MBN), and P (MBP) contents were measured following the chloroform fumigation extraction method given by Brookes et al. [38] and Vance et al. [39].

#### 2.4. Measurement of Soil EEA

Activities of C-acquisition enzymes ( $\alpha$ -glucosidase (AG),  $\beta$ -1,4-glucosidase (BG), and  $\beta$ -1,4-xylosidase (BX)), of the N-acquisition enzymes ( $\beta$ -1,4-N-acetylglucosaminidase (NAG) and L-leucine aminopeptidase (LAP)), and of the P-acquisition enzyme alkaline phosphatase (AP) were assessed by fluorometry according to DeForest [40] with substrates linked to fluorescent molecules and using a special buffer solution which buffered the enzyme-substrate solutions in a similar pH range as occurred at the study sites [41]. A 4-methylumbelliferone and phosphate buffer solution (pH 8.0) was used to quantify AG, BG, BX, NAG, and AP, whereas 7-amino-4-methylcoumarin and tris(hydroxymethyl)aminomethane buffer (pH 8.0) were used to quantify LAP. The detailed EEA measurement procedures have been described previously [16]. All enzyme reactions were incubated in the dark at 25 °C for 2 h before measurement at 355 nm excitation wavelength and 460 nm emission wavelength using a multimode microplate reader (infinite M1000PRO; Tecan, Männedorf, Switzerland).

#### 2.5. Statistical Analyses

Ratios of soil extracellular C-, N-, and P-acquisition enzymes were considered to represent the ratio of EEAs directed toward acquiring C, N, and P from soil. They were calculated using data based on untransformed proportional activities according to the following equations (Equations (1)–(3)):

$$C : N \text{ acquisition} = (AG + BG + BX) / (NAG + LAP) \quad (1)$$

$$C : P \text{ acquisition} = (AG + BG + BX) / (AP) \quad (2)$$

$$N : P \text{ acquisition} = (NAG + LAP) / (AP) \quad (3)$$

where C:N acquisition ( $EEA_{C:N}$ ) is the ratio of soil extracellular C-acquisition enzymes to N-acquisition enzymes; C:P acquisition ( $EEA_{C:P}$ ) is the ratio of soil extracellular C-acquisition enzymes to P-acquisition enzyme; and N:P acquisition ( $EEA_{N:P}$ ) is the ratio of soil extracellular N-acquisition enzymes to P-acquisition enzyme [42,43].

Soil microbial nutrient status and metabolic limitations, as inferred from soil extracellular coenzymatic stoichiometry, were assessed by calculating vector length (VL) and vector angle (VA) of extracellular enzymes as well as the threshold elemental ratios (TERs). VL represents microbial C limitation, with longer VL indicating stronger microbial C limitation, and VA represents microbial N and P limitation, with a VA larger or smaller than 45° indicating microbial P limitation and N limitation, respectively [44].  $TER_{C:N}$  and  $TER_{C:P}$  represent the elemental ratio at which metabolic control of microbial metabolic limitation switches between C limitation and nutrient (N or P) limitation [19,45]. VL, VA, and TER were calculated using the following equations (Equations (4)–(7)):

$$VL = \sqrt{(C : P \text{ acquisition})^2 + (C : N \text{ acquisition})^2} \quad (4)$$

$$VA = \text{degrees}(\text{ATAN2}((C : P \text{ acquisition}), (C : N \text{ acquisition}))) \quad (5)$$

$$TER_{C:N} = (C : N \text{ acquisition}) \times MB_{C:N} / n_0 \quad (6)$$

$$TER_{C:P} = (C : P \text{ acquisition}) \times MB_{C:P} / p_0 \quad (7)$$

where  $MB_{C:N}$  and  $MB_{C:P}$  are the MBC to MBN and MBC to MBP ratios, respectively; and  $n_0$  and  $p_0$  are dimensionless normalization constants that represent the intercepts of  $\ln(AG + BG + BX)$  vs.  $\ln(NAG + LAP)$  and  $\ln(AG + BG + BX)$  vs.  $\ln(AP)$ , respectively.

A one-way analysis of variance (ANOVA) followed by Fisher's least significant difference test at  $p < 0.05$  was performed to test the differences in soil EEAs, microbial metabolic limitation indicators, and environmental variables (physicochemical and microbial biomass properties) across the *S. canadensis* invasion gradient. Redundancy analysis (RDA) and a permutational multivariate ANOVA (PERMANOVA) were performed to test site differences in soil EEAs and microbial metabolic limitation indicators in relation to environmental variables. A variation partitioning analysis (VPA) was performed using the RDA results to further assess the relative importance of soil environmental variables on soil EEAs and microbial metabolic limitation indicators. Partial least squares path modeling (PLS-PM) was performed to evaluate possible pathways by which variables affect soil microbial metabolic limitation indicators following *S. canadensis* invasion. ANOVA, RDA, and PLS-PM were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA), CANOCO version 5.0 (Microcomputer Power, Inc., Ithaca, NY, USA), and Amos in IBM SPSS version 24.0 (SPSS Inc., Chicago, IL, USA), respectively, and PERMANOVA and VPA were performed using R software version 4.0.2 (R Core Team [46]).

### 3. Results

#### 3.1. Soil Physicochemical and Microbial Biomass Properties

Soil physicochemical and microbial biomass properties differed across the invasion gradient of *S. canadensis*. Soil moisture (SM) was 20.85% and 15.51% lower at the *S. canadensis*-dominated (SD) site than at the co-dominated (CD) and *P. australis*-dominated (PD) sites, respectively ( $p < 0.05$ ). Soils at the SD site displayed significantly higher CEC and DOC content than CD and PD sites' soils ( $p < 0.01$ , each). Soils were alkaline across the invasion gradient of *S. canadensis*. The pH of soils was greatest at the SD site and lowest at the CD site. Compared to the PD site, SD and CD sites showed changes in STC by  $-17.83\%$  and  $7.79\%$  and in STN by  $-16.48\%$  and  $8.01\%$ , respectively. STP was significantly increased by  $20.21\%$  and  $4.46\%$  at SD and CD sites, respectively, resulting in significant alterations in soil resource ratios of STC to STP by  $-31.48\%$  and  $3.00\%$  and in STN to STP by  $-30.64\%$  and  $2.95\%$  at SD and CD sites, respectively, compared to the PD site. Soil microbial biomass properties showed different trends across the invasion gradient of *S. canadensis*, which were, however, not statistically significant (Table 1).

**Table 1.** Soil properties across the *S. canadensis* invasion gradient, presented as mean  $\pm$  standard error ( $n = 4$ ).

Parameters	F	p	Invasion Gradient		
			SD	CD	PD
SM ( $w/w\%$ )	4.42	*	19.36 $\pm$ 0.57b	24.46 $\pm$ 2.01a	22.91 $\pm$ 0.51ab
pH	3.07	ns	8.30 $\pm$ 0.01	8.20 $\pm$ 0.05	8.27 $\pm$ 0.02
CEC ( $\text{cmol}_c \text{ kg}^{-1}$ )	9.48	**	10.55 $\pm$ 0.02a	10.38 $\pm$ 0.07b	10.28 $\pm$ 0.03b
SOM ( $\text{mg g}^{-1}$ soil)	1.16	ns	13.04 $\pm$ 0.92	16.81 $\pm$ 2.85	14.78 $\pm$ 0.49
DOC ( $\times 10^{-1} \text{ mg C g}^{-1}$ soil)	13.12	**	2.86 $\pm$ 0.04a	2.66 $\pm$ 0.09b	2.41 $\pm$ 0.03c
SOC ( $\text{mg C g}^{-1}$ soil)	1.16	ns	7.56 $\pm$ 0.53	9.75 $\pm$ 1.65	8.57 $\pm$ 0.29
STC ( $\text{mg C g}^{-1}$ soil)	1.41	ns	10.82 $\pm$ 0.52	14.19 $\pm$ 2.21	13.17 $\pm$ 1.09
SIN ( $\times 10^{-3} \text{ mg N g}^{-1}$ soil)	1.31	ns	2.24 $\pm$ 0.79	6.85 $\pm$ 0.78	7.67 $\pm$ 4.28
STN ( $\text{mg N g}^{-1}$ soil)	1.56	ns	0.89 $\pm$ 0.04	1.15 $\pm$ 0.17	1.06 $\pm$ 0.03
SAP ( $\times 10^{-2} \text{ mg P g}^{-1}$ soil)	6.03	*	2.40 $\pm$ 0.16a	2.69 $\pm$ 0.27a	1.79 $\pm$ 0.09b
STP ( $\times 10^{-1} \text{ mg P g}^{-1}$ soil)	7.9	*	8.23 $\pm$ 0.24a	7.15 $\pm$ 0.32b	6.85 $\pm$ 0.20b
ST <sub>C:N</sub>	0.03	ns	12.21 $\pm$ 0.23	12.35 $\pm$ 0.22	12.34 $\pm$ 0.65
ST <sub>C:P</sub>	4.57	*	13.12 $\pm$ 0.25b	19.72 $\pm$ 2.74a	19.15 $\pm$ 1.10a
ST <sub>N:P</sub>	5.05	*	1.08 $\pm$ 0.03b	1.60 $\pm$ 0.22a	1.55 $\pm$ 0.02a
MBC ( $\times 10^{-1} \text{ mg C g}^{-1}$ soil)	1.16	ns	6.40 $\pm$ 2.30	3.65 $\pm$ 1.86	8.84 $\pm$ 2.95
MBN ( $\times 10^{-2} \text{ mg N g}^{-1}$ soil)	1.31	ns	2.45 $\pm$ 0.44	1.28 $\pm$ 0.44	1.84 $\pm$ 0.62
MBP ( $\times 10^{-2} \text{ mg P g}^{-1}$ soil)	1.09	ns	1.36 $\pm$ 0.27	1.56 $\pm$ 0.41	2.12 $\pm$ 0.43

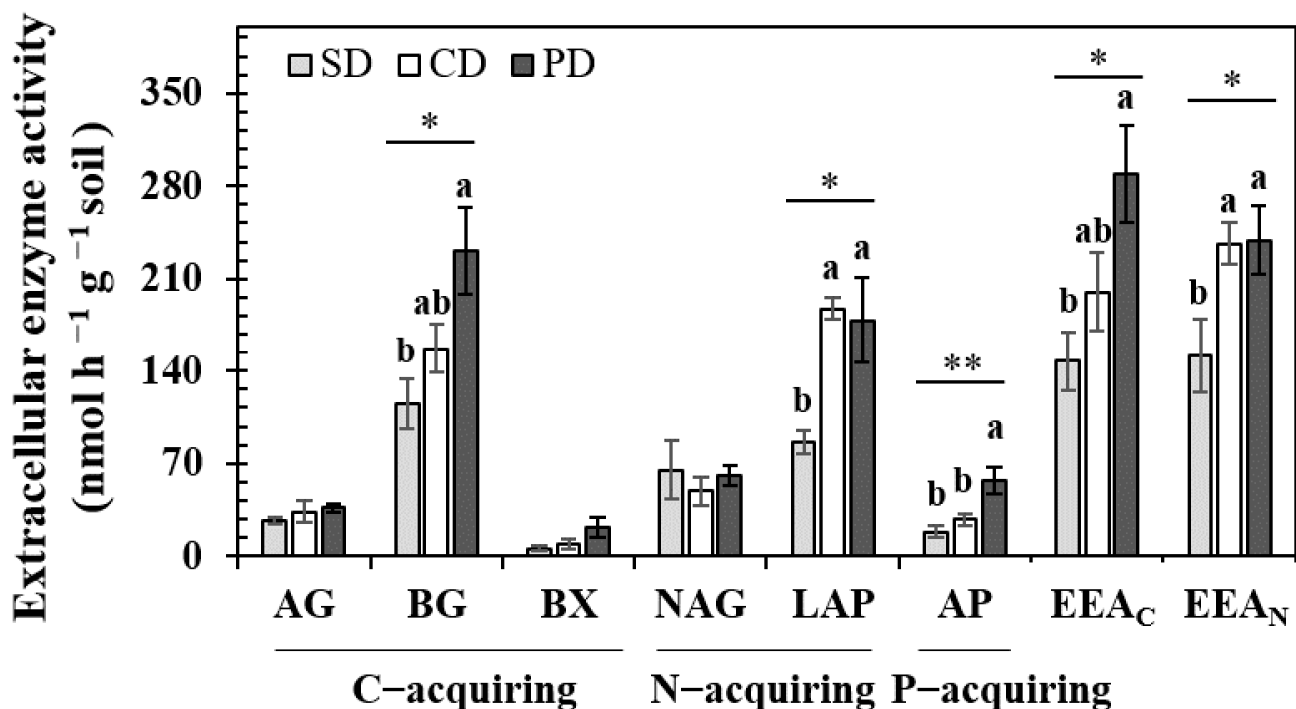
Table 1. Cont.

Parameters	F	p	Invasion Gradient		
			SD	CD	PD
MB <sub>C:N</sub>	0.89	ns	27.49 ± 9.81	32.25 ± 9.02	58.79 ± 28.04
MB <sub>C:P</sub>	0.7	ns	50.70 ± 14.71	23.82 ± 7.20	53.94 ± 29.96
MB <sub>N:P</sub>	0.62	ns	2.58 ± 1.28	1.51 ± 1.00	1.08 ± 0.47

SD = *S. canadensis*-dominated site; CD = co-dominant (*S. canadensis* and *P. australis*) site; PD = *P. australis*-dominant site; SM = soil moisture; CEC = cation exchange capacity; SOM = soil organic matter; DOC = dissolved organic carbon (C); SOC = soil organic C; STC = soil total C; SIN = soil inorganic nitrogen (N); STN = soil total N; STP = soil total phosphorus (P); SAP = soil available P; ST<sub>C:N</sub> = ratio of STC to STN; ST<sub>C:P</sub> = ratio of STC to STP; ST<sub>N:P</sub> = ratio of STN to STP; MBC = soil microbial biomass C; MBN = soil microbial biomass N; MBP = soil microbial biomass P; MB<sub>C:N</sub> = ratio of MBC to MBN; MB<sub>C:P</sub> = ratio of MBC to MBP; MB<sub>N:P</sub> = ratio of MBN to MBP. Different letters denote significant differences ( $p < 0.05$ ) across the invasion gradient of *S. canadensis*. ns = not significant at the level of  $p > 0.05$ ; \* = significant at the level of  $p < 0.05$ ; and \*\* = significant at the level of  $p < 0.01$ .

### 3.2. Soil EEAs and Microbial Metabolic Limitation Indicators

*S. canadensis* invasion induced significant differences in BG ( $p < 0.05$ ), LAP ( $p < 0.05$ ), and AP activities ( $p < 0.01$ ), in C-acquisition enzyme activities (including AG, BG, and BX;  $p < 0.05$ ), N-acquisition enzyme activities (including NAG and LAP;  $p < 0.05$ ), and in the ratio of C-acquisition to P-acquisition enzymes ( $p < 0.05$ ) (Figure 1; Table 2). Sites with more *S. canadensis* invasion tended to have lower soil enzyme levels. Specifically, activities of all individual enzymes and most of C- and N-acquisition enzymes were reduced with the only exception of NAG activity, which was increased for the SD site (Figure 1). Compared to the PD site, the ratios of C-acquisition to P-acquisition enzymes significantly increased by 64.75% and 39.97% ( $p < 0.05$ ) at SD and CD sites, respectively (Table 2).



**Figure 1.** Variations in soil EEAs involved in carbon (C)-, nitrogen (N)-, and phosphorus (P)-acquiring across the *S. canadensis* invasion gradient sites ( $n = 4$ ). Vertical bars indicate the standard error. SD = *S. canadensis*-dominated site; CD = co-dominant (*S. canadensis* and *P. australis*) site; PD = *P. australis*-dominant site; AG =  $\alpha$ -glucosidase; BG =  $\beta$ -1,4-glucosidase; BX =  $\beta$ -1,4-xylosidase; NAG =  $\beta$ -1,4-N-acetylglucosaminidase; LAP = L-leucine aminopeptidase; AP = alkaline phosphatase; EEA<sub>C</sub> = C-acquisition enzymes; EEA<sub>N</sub> = N-acquisition enzymes. Different letters denote significant differences ( $p < 0.05$ ) across the *S. canadensis* invasion gradient sites. \* = significant at the level of  $p < 0.05$ , and \*\* = significant at the level of  $p < 0.01$ .

**Table 2.** Soil extracellular ecoenzymatic stoichiometry indicators across the *S. canadensis* invasion gradient, presented as mean  $\pm$  standard error ( $n = 4$ ).

Parameters	F	<i>p</i>	Invasion Gradient		
			SD	CD	PD
EEA <sub>C:N</sub>	1.63	ns	1.02 $\pm$ 0.14	0.84 $\pm$ 0.09	1.27 $\pm$ 0.24
EEA <sub>C:P</sub>	5.07	*	8.66 $\pm$ 1.01a	7.36 $\pm$ 0.50ab	5.26 $\pm$ 0.69b
EEA <sub>N:P</sub>	2.93	ns	9.30 $\pm$ 2.13	9.29 $\pm$ 1.72	4.45 $\pm$ 0.73
VL	4.81	*	8.73 $\pm$ 1.00a	7.41 $\pm$ 0.49ab	5.42 $\pm$ 0.70b
VA ( $^{\circ}$ )	5.97	*	7.12 $\pm$ 1.54b	6.64 $\pm$ 0.90b	13.63 $\pm$ 2.11a
TER <sub>C:N</sub>	1.16	ns	1.80 $\pm$ 0.66	1.74 $\pm$ 0.47	5.99 $\pm$ 3.84
TER <sub>C:P</sub>	1.14	ns	4.34 $\pm$ 1.48	1.74 $\pm$ 0.62	2.69 $\pm$ 1.42

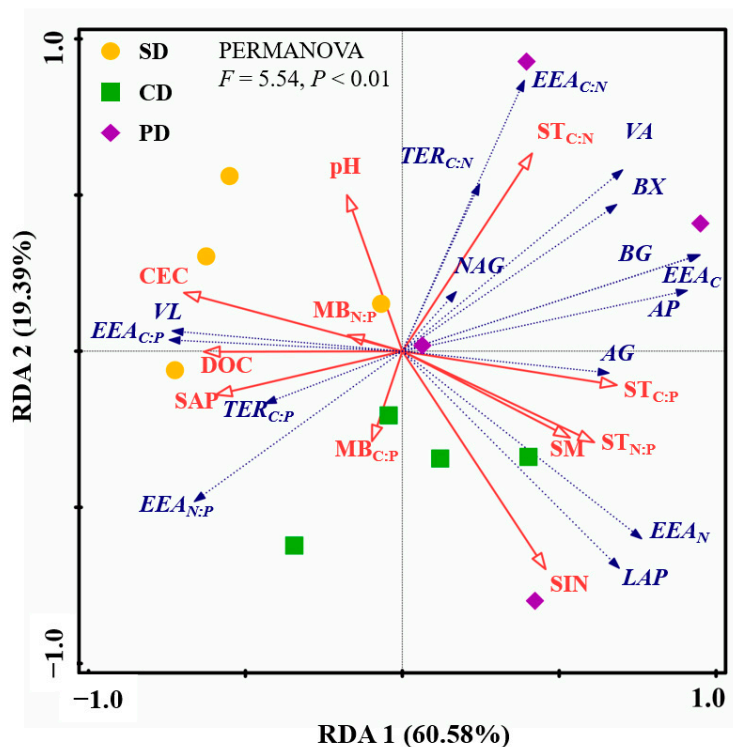
EEA<sub>C:N</sub> = the ratio of soil extracellular C-acquisition enzymes to N-acquisition enzymes; EEA<sub>C:P</sub> = the ratio of soil extracellular C-acquisition enzymes to P-acquisition enzymes; EEA<sub>N:P</sub> = the ratio of soil extracellular N-acquisition enzymes to P-acquisition enzymes; VL = vector length; VA = vector angle; TER<sub>C:N</sub> = threshold elemental ratio of C to N; TER<sub>C:P</sub> = threshold elemental ratio of C to P. Different letters denote significant differences ( $p < 0.05$ ) across the invasion gradient of *S. canadensis*. ns = not significant at the level of  $p > 0.05$ ; \* = significant at the level of  $p < 0.05$ .

The patterns of soil microbial metabolic limitation indicators as reflected by extracellular ecoenzymatic stoichiometry differed across the invasion gradient of *S. canadensis*. VLs and VAs ranged from 5.42 to 8.73 and from 6.64 $^{\circ}$  to 13.63 $^{\circ}$ , respectively. At the SD site, VL was significantly higher (by 61.11%) than at the PD site, whereas VA was significantly lower (by 47.80%) than at the PD site ( $p < 0.05$ , each). All VAs were smaller than 45 $^{\circ}$ , and no significant differences in TER (TER<sub>C:N</sub> and TER<sub>C:P</sub>) were observed. TER<sub>C:N</sub> at the SD site was 3.33-fold lower than that at the PD site, whereas TER<sub>C:P</sub> showed the opposite pattern with 1.61-fold higher values at sites dominated by *S. canadensis* than the ones dominated by *P. australis* (Table 2). Taken together, the results suggest considerable C and N limitation of soil microbial metabolism at *S. canadensis*-invaded sites.

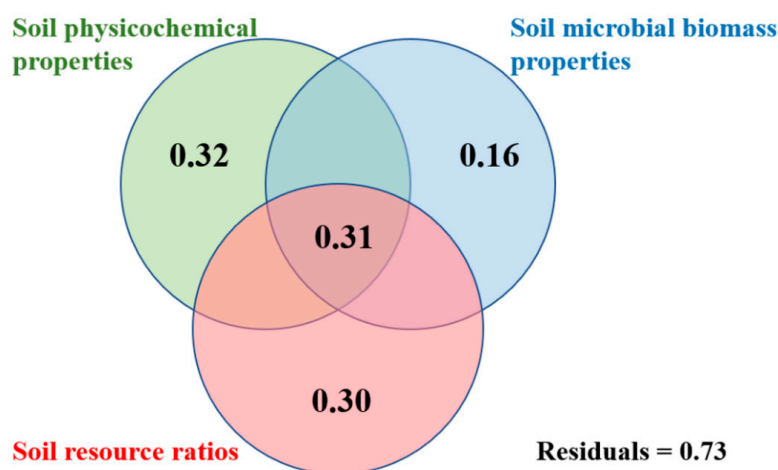
### 3.3. Relationships of Soil EEAs, Soil Microbial Metabolic Limitation Indicators, and Soil Properties

RDA and PERMANOVA results show spatial variability in all EEAs and microbial metabolic limitation indicators, and RDA1, which accounted for 60.58% of the variability-distinguished samples across the invasion gradient of *S. canadensis* ( $p < 0.01$ ; Figure 2). The VPA suggested that soil physicochemical properties, resource ratios, and microbial biomass properties explained 32%, 30%, and 16% of variation, respectively (Figure 3). CEC, DOC, the ratio of STC to STP, and the ratio of STN to STP together accounted for 66.4% of the variability; these factors are therefore considered as key properties.

The PLS-PM analysis demonstrated that the alterations in soil properties and resource ratios induced by *S. canadensis* invasion affected EEAs and ultimately influenced soil microbial metabolic limitation indicators (Figure 4). The effects of the ratio of C-acquisition to P-acquisition enzymes on VL ( $-0.604$ ) and VA ( $0.984$ ) showed the reverse pattern. Moreover, the ratio of C-acquisition to P-acquisition enzymes was found to be a direct driver of microbial metabolic limitation variation at all sites.

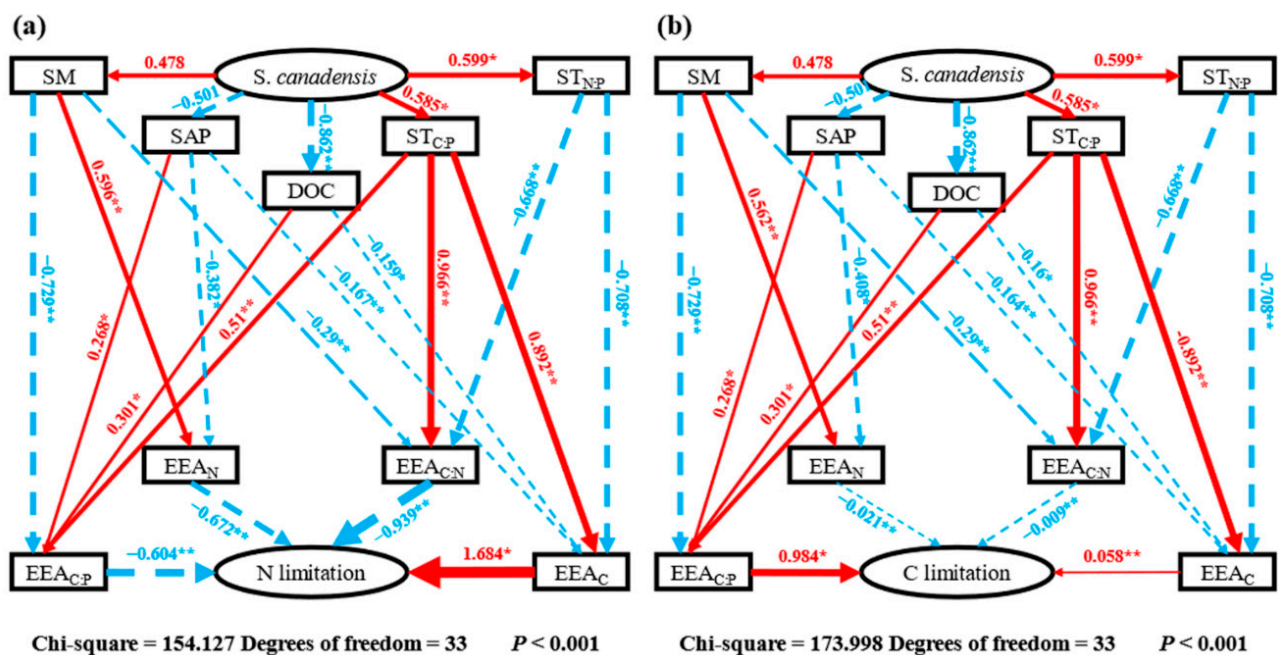


**Figure 2.** Result of redundancy analysis (RDA) and permutational multivariate analysis of variance (PERMANOVA) based on soil EEAs, microbial metabolic limitation indicators, and soil properties. EEA<sub>C:N</sub> = the ratio of soil extracellular C-acquisition enzymes to N-acquisition enzymes; EEA<sub>C:P</sub> = the ratio of soil extracellular C-acquisition enzymes to P-acquisition enzymes; EEA<sub>N:P</sub> = the ratio of soil extracellular N-acquisition enzymes to P-acquisition enzymes; VL = vector length; VA = vector angle; TER<sub>C:N</sub> = threshold elemental ratio of C to N; TER<sub>C:P</sub> = threshold elemental ratio of C to P; SM = soil moisture; CEC = cation exchange capacity; DOC = dissolved organic C; SIN = soil inorganic N; SAP = soil available P; ST<sub>C:N</sub> = ratio of STC to STN; ST<sub>C:P</sub> = ratio of STC to STP; ST<sub>N:P</sub> = ratio of STN to STP; MB<sub>C:P</sub> = ratio of MBC to MBP; MB<sub>N:P</sub> = ratio of MBN to MBP.



**Figure 3.** Result of variation partitioning analysis (VPA) showing the effects of soil physicochemical properties, microbial biomass properties, and resource ratios on soil EEAs and microbial metabolic limitation indicators. Soil physicochemical properties include SM, pH, CEC, DOC, and SAP; microbial biomass properties include ratio of MBC to MBP and ratio of MBN to MBP; resource ratios include ratio of STC to STN, ratio of STC to STP, and ratio of STN to STP.





**Figure 4.** Cascading relationships of (a) microbial metabolism C limitation and (b) microbial metabolism N limitation with soil properties and EEAs. Partial least squares path modeling (PLS-PM) disentangling major pathways of the influences of soil properties and EEAs on microbial metabolism C limitation and microbial metabolism N limitation. Red and blue arrows indicate positive and negative flows of causality; \* = significant at the level of  $p < 0.05$ , and \*\* = significant at the level of  $p < 0.01$ . Numbers on the arrow indicate significant standardized path coefficients.

## 4. Discussion

### 4.1. The Effects of *S. canadensis* Invasion on Soil EEAs

*S. canadensis* invasion was originally hypothesized to increase the activities of soil extracellular enzymes due to its faster growth and higher productivity leading to increased litter input into the soil [27,28]. In contrast to this prediction, *S. canadensis* invasion sites showed decreased C-, N-, and P-acquisition enzymatic activities (apart from NAG activity; Figure 1). Furthermore, soil EEAs were positively or negatively correlated with soil physicochemical properties and resource ratios (Figures 2 and 3 and Figure S1), which may suggest that suppression of EEAs was due to changes in the soil nutrient status. These conclusions are in line with those of other studies on alien invasive plants, which exhibited that changes in C-, N-, and P-acquisition enzymes were associated with changes in soil available nutrients, indicating that limitations and imbalances of nutrients can partially underlie production of soil enzymes and affect their activity [18,47,48]. Owing to their fast growth, alien invasive plants may outcompete both native vegetation and soil microbes through rapid uptake and use of soil nutrients [5,26]. Thus, *S. canadensis* invasion may aggravate nutrient limitations and imbalances in the soil microenvironment and in soil microbes, which may elicit further direct and indirect adverse effects on microbial resource acquisition, thereby suppressing soil microbial growth and enzyme productions [47,49].

A previous study has shown that the activities of C-, N-, and P-acquisition enzymes can be inhibited by interactions with compounds released by *S. canadensis* (Kim et al., 2018). SOM is a primary substrate of enzymatic activities; however, *S. canadensis* invasion apparently did not induce changes in SOM at the sites of the current study (Table 1), which suggests that differences in enzyme activities across the invasion gradient of *S. canadensis* may be due to plant compounds in the soil and/or interactions between plant secondary compounds and functional groups of microbes [11,25]. Plant secondary compounds in soil are subjected to enzymatic degradation, and their constituents are integrated during enzyme synthesis [50]. The previous study at the same field sites showed that the cellulose

in leaves of *S. canadensis* was lower than that in leaves of *P. australis* (Table S1; data from Hu [51]). Less abundant cellulose as a hydrolysis substrate for soil microbes may inhibit microbial production of cellulases such as BG (Figure 1). Additionally, previous studies found that allelopathic exudates from *S. canadensis* may inhibit native plant growth, and they also induce changes in specific soil microbe functional groups, inhibit the activity of soil microbes, and subsequently suppress enzymatic activities [16,17,29,48,52,53]. This was in line with the higher phenol and flavone concentrations in *S. canadensis* leaves (Table S1; data from Hu [51]). Thus, soil nutrient composition may vary between vegetation communities as a consequence of differential effects on soil extracellular enzymes.

#### 4.2. The Effects of *S. canadensis* Invasion on Soil Microbial Metabolic Limitations

It was predicted that *S. canadensis* invasion would induce changes in microbial nutrient status and metabolic limitations, which should be reflected in soil extracellular ecoenzymatic stoichiometry. This hypothesis was supported by significant variation in soil extracellular ecoenzymatic stoichiometry, which revealed nutritional limitations of microbial metabolism (Table 2). The opposite trend was observed regarding  $TER_{C:N}$  and  $TER_{C:P}$ , which suggested that *S. canadensis* invasion increased the sensitivity of soil microbes to nutrient limitation. All VA points were below the 1:1-line (with VAs smaller than  $45^\circ$ ), indicating that N was a limiting factor for soil microbes at all sites. However, as VL became greater at *S. canadensis*-invaded sites, the microbial N limitation would gradually convert to C limitation, which resulted in a reduced microbial N assimilation. Altered microbial N limitation can substantially influence the growth and metabolism of microbes because microbes must maintain the homeostasis and requirements of nutrients, thereby reducing competition for N between plants and soil microbes to facilitate successful invasion [32].

As the availability and stability of nutrients are likely the fundamental drivers of both plant and microbial community succession, changes in soil nutrient status after *S. canadensis* invasion may be the predominant mechanism underlying the increasing microbial C and N limitation due to the nutrient requirements of microbial homeostasis. This assumption is supported by the RDA and PLS-PM results (Figure 4 and Figure S1). Competition for soil nutrients between invasive and native plants and between plants and soil microbes may cause nutrient limitations and imbalance [45]. Meanwhile, *S. canadensis* invasion is speculated to alter soil hydrology and nutrient input and thereby affect nutrient availability. For example, significantly higher P content (regarding both STP and SAP) at *S. canadensis*-dominated sites may indirectly affect C and N mineralization due to co-metabolism processes with P, which in the present study was supported by the observed significant differences in DOC, the ratio of STC to STP and of STN to STP (Table 1). The induced limitations and imbalance of soil nutrients further restrained enzymes (Figure 1 and Table 2), as soil microbes can regulate enzyme production and ecoenzymatic stoichiometry, particularly so in nutrient-limited microenvironments [19]. Nutrient requirements of microbial homeostasis modulated their response and metabolism to the soil nutrient deficiency, leading to a relative microbial C and/or N limitation. Consequently, soil microbes may attempt to increase acquisition of limiting C and N to maintain stoichiometric homeostasis and facilitate growth under nutrient-limited conditions [21,45,54], as may be induced by *S. canadensis* invasion.

#### 4.3. Implication of *S. canadensis* Invasion Effects on Soil EEAs and Microbial Metabolic Limitations

In ecologically sensitive areas (e.g., natural riparian habitats), changes in vegetation community succession may alter the hydrologic functioning and may affect soil nutrient input and microbial communities, thereby changing the soil biogeochemical nutrient cycling processes [14,22]. Previous studies suggested that short-term effects of vegetation community changes on soil physiochemical properties may not be as strong as long-term effects; however, vegetation community changes may affect soil extracellular enzymes due to altered plant nutrient uptake and changed soil microbiomes [55]. Corresponding mechanisms were observed in the present study, as *S. canadensis* invasion appeared to sig-

nificantly affect several soil physiochemical properties, EEAs, and soil microbial metabolic limitations (Figures 1 and 2; Tables 1 and 2).

Variability in soil available nutrients may be the predominant mechanism underlying changes in soil EEAs and microbial metabolic limitations following *S. canadensis* invasion. *S. canadensis* invasion will likely induce biogeochemical modifications in many areas. The replacement of *P. australis* by *S. canadensis* will result in nutrient-limited microenvironments by competition and continuous input of specific metabolic substrates into the soil. The deficiency and imbalance of soil available nutrients, as was the case in the present study, may compel soil microbiomes to initially break down complex substrates to meet nutrient demands; however, such complex substrates may require more microbial enzymatic steps for degradation which further decreases conversion efficiency of nutrient [17,27]. Therefore, *S. canadensis* invasion is likely to alter nutrient cycling and decrease the activity (e.g., enzyme production) and growth of soil microbiomes.

## 5. Conclusions

*S. canadensis* invasion appeared to be associated with markedly reduced C-, N-, and P-acquiring enzyme activities (apart from NAG) and with changes in soil microbial metabolic limitations. These shifts are fully paralleled by the shifts in soil available nutrients induced by *S. canadensis* invasion. The present results suggest *S. canadensis* invasion can affect the C-, N-, and P-acquiring enzyme and the soil microbial metabolisms which in turn alter biogeochemical cycling processes in previously *P. australis*-dominated riparian habitats, and a positive, self-reinforcing feedback mechanism of nutrient cycling may facilitate successful *S. canadensis* invasion and persistence.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/su13073768/s1>, Table S1: Leaf characteristic of Canada goldenrod (*Solidago canadensis* L.) and common reed (*Phragmites australis*) among the study sites, presented as mean  $\pm$  standard error, Figure S1: Heat map of correlation among soil properties, EEAs, and microbial metabolic limitation indicators among the study areas.

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