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Original Research

Nitrogen Deposition Enhanced the Effect of *Solidago Canadensis* Invasion on Soil Microbial Metabolic Limitation and Carbon Use Efficiency

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Abstract

Solidago canadensis, considered one of the most destructive invasive species of the 21st century, has inflicted severe ecological damage globally. Nevertheless, the precise mechanisms through which nitrogen deposition impacts the metabolism of soil microorganisms across various vegetation communities remain largely unexplored. Consequently, this study sought to elucidate patterns of change in microbial metabolic constraints and carbon use efficiency across six distinct vegetation communities, aiming to uncover the carbon cycling process within soil ecosystems. Our findings reveal that multi-vegetation communities exhibit greater resilience to the incursion of *Solidago canadensis* than their single-vegetation counterparts, demonstrating weaker microbial carbon constraints and higher carbon use efficiency. Furthermore, a positive correlation was determined between microbial carbon use efficiency and carbon constraints. Driven by soil nutrients, nitrogen deposition synergistically interacts with *Solidago canadensis*, thereby influencing soil microbial carbon use efficiency. Thus, our experiment provides an initial perspective on the variations in metabolic limitations and carbon use efficiency amongst soil microbes across different vegetation communities. Such insights hold potential implications for future research focused on the feedback and responses of carbon cycles within varied vegetation community ecosystems to invasions in the context of increasing nitrogen deposition.

Keywords: alien plant invasion, Canada goldenrod, extracellular ecoenzymic stoichiometry, native vegetation community diversity

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Introduction

Invasive alien plant species pose significant threats to natural ecosystems, leading to a loss of biodiversity [1, 2] and a decline in the abundance of native species within invaded areas [3]. Thus, the mechanisms underlying the successful invasion of these alien plants have emerged as a long-term focal point within the environmental field, particularly in relation to the relationship between alien invasive plants and ecosystem carbon cycling. Existing research suggests that invasive alien plants are transforming Earth's ecosystems and how they interact the carbon cycling, often modifying the carbon cycle via potential interrelated mechanisms, most notably through substantial biomass input into the soil [4]. However, how the interaction between invasive alien plants and the invaded vegetation communities shapes the ecosystem's carbon cycle remains unclear, with most studies primarily focusing on the high growing ability of invasive alien species and its allelopathic impact [5, 6]. This study aims to investigate the various effects on the ecosystem's carbon turnover that result from the introduction of alien plants into different vegetative communities, as well as their corresponding mechanistic foundations.

Along with invasive alien plants, nitrogen deposition is a hot problem that has received much academic interest. Its origin is primarily attributed to human activities such as fossil fuel burning, usage of nitrogen fertilizers, and livestock industry development, which together produce substantial quantities of nitrogen compounds, thus escalating atmospheric nitrogen deposition trends [7]. This continual increase in nitrogen input has altered nutrient cycling in natural ecosystems [8], triggering changes in the invasion of invasive plant species and plant physiological and ecological behaviours [9]. Consequently, nitrogen deposition has become an essential topic of scientific inquiry within ecological research, seeking to elucidate its positive or negative impacts on invasive plant species and their corresponding mechanistic drivers. Furthermore, the increasing nitrogen input globally could result in higher soil nitrogen content, which could change microbial carbon use efficiency and impact the global carbon cycle due to the interconnectedness of the carbon-nitrogen cycle and the effects of nitrogen accessibility on microbial metabolic processes [10].

Existing studies suggest that soil microbial metabolic constraints indicate soil resource availability [11]. Based on the ecological stoichiometry and metabolic theory developed by [12, 13], these metabolic constraints can be evaluated through the stoichiometry of extracellular ecological enzymes [12, 13]. Another essential metric for assessing microbial metabolism is microbial carbon use efficiency, which signifies the ratio of carbon employed for physiological growth to total microbial carbon absorption [14, 15]. Higher soil microbial carbon use efficiency indicates an elevated efficiency of microbes in converting soil nutrients into their biomass, which could enhance the potential for soil carbon sequestration. Conversely, lower carbon use efficiency implies that a significant amount of carbon is released into the atmosphere via microbial respiration, potentially diminishing soil carbon storage [16].

Solidago canadensis (Canada goldenrod), initially introduced in North America as a horticultural plant in 1935, has emerged as a severely detrimental invasive species to native ecosystems in China [17, 18]. This study aims to evaluate the response of soil microbes within diverse vegetation communities to the invasion of *Solidago canadensis* within the context of nitrogen deposition. The primary objective is to examine the alterations in soil microbial metabolic patterns, identifying the driving factors behind these changes. To validate this, we implemented a pot experiment simulating the invasion of *Solidago canadensis* under conditions of nitrogen deposition. The goal is to unveil how the *Solidago canadensis* invasion affects soil microbes within different vegetation communities, which influences the carbon cycling process in soil ecosystems. In order to examine the patterns of changes in metabolic constraints and carbon use efficiency in invaded vegetative communities as a result of alien plant invasion and to uncover the underlying causes of this trend, we want to use soil microbial metabolic constraints and carbon use efficiency. The hypotheses of this study are as follows: (1) Nitrogen deposition will end up resulting in a reduction in soil microbial metabolic limitations and carbon use efficiency; (2) *Solidago canadensis* invasion has less impact on microbial metabolic constraints and carbon use efficiency in single vegetation communities than it does on multiple vegetation communities; and (3) Soil moisture and nutrient distribution are the main factors affecting changes in soil microbial metabolic constraints and carbon use effectiveness.

Material and Methods

Study Region Description and Design

The study was conducted in a greenhouse located at the greenhouse of Jiangsu University (32°21'N, 119°52'E). In 2020, the annual air temperature and natural precipitation were 16.9ºC and 1209.9 mm, respectively. The experiment period is from June to December 2020. The soil used for the experiment was collected from slopes within the campus grounds. Our research subjects included the *Solidago canadensis*, local plant *Artemisia argyi*, and *Solidago decurrens*, and we employed a method where *Solidago canadensis* invaded different vegetation communities under two conditions: with nitrogen application (N_5) and without nitrogen (N_0) application. In this experiment, nitrogen sedimentation treatment was simulated by artificially adding nitrogen solution. The simulated nitrogen sedimentation concentration was 5 g N m^{-2} yr^{-1} (N_5), and the nitrogen solution was a mixed solution with a ratio of 3.5:3.5:3 of $NaNO_3$:NH_4Cl:CH_4N_2O. Nitrogen was added

three times (June 10, June 25, July 10), and the same amount of water was added to the control treatment. The height of each pot is 25 cm, the diameter of the base is 19 cm, and the diameter of the top is 24 cm. Each pot is put into 5 kg of dry soil. Soil moisture is maintained at 60% of the maximum moisture content. Each pot contained five plants, including one invasive plant and four local ones, make sure plants get plenty of light. The single vegetation community consisted of either four *Artemisia argyi* (A) or four *Solidago decurrens* (S), while the multiple vegetation community consisted of a mix of two *Artemisia argyi* and two *Solidago decurrens* (AS). Thus, there were a total of six treatments and three repetitions, with the total of 18 pots. All the plants were self-grown, and only seedlings with consistent vigor were chosen for post-germination transplanting into pots.

Soil Samples Collection and Preparation

Five randomly selected points inside each pot were used to collect and evenly mix soil samples from a depth of 0 to 15 cm. Prior to further analysis, these soil samples were passed through a 2 mm sieve to remove discernible stones and plant remnants. The samples were subsequently kept at 4ºC in a refrigerator while the extracellular enzyme activity, microbial biomass, and soil inorganic nitrogen were all analyzed.

Soil Properties Measurement

The drying technique was used to measure soil moisture, while spectrophotometry was used to measure the inorganic nitrogen in the soil [19, 20]. In detail, soil moisture was measured as mass loss after oven-drying at 105ºC for 72 h. After sifting, fresh soil equivalent to 5 g air-dried soil weight was added to the fresh soil in a 50 mL centrifuge tube, 25 mL 2 M potassium chloride solution was added, and the solution was shaken at room temperature for 40 min (200 r/min), filtered, and the filtrate was taken for use (soil leaching extract). 1.5 mL of the filtered soil leaching extract was added into a 15 mL centrifuge tube with 0.5 mL EDTA masking agent, 2 mL color developer, 7.5 mL ultra-pure water and 1mL buffer solution in turn. The water bath was heated for 30 min (37ºC), and the absorbance value was measured at the wavelength of 667 nm to calculate the content of ammonium nitrogen per unit mass of soil. After filtration, 0.06 mL of soil extract was placed in a 5 mL colorimetric dish, 3 mL of color developer was added, and the absorbance value was determined at 540 nm wavelength for 15 h, and the nitrate nitrogen content per unit mass of soil was calculated. The soil's dissolved organic carbon and nitrogen levels were determined through the water extraction method [21]. After sifting, fresh soil equivalent to 5 g air-dried soil weight was added into a 50 mL centrifuge tube, 25 mL 0.5M potassium sulfate solution was added, and then shaken at room temperature for 40 min (200 r/min), filtered, and the concentration of dissolved carbon and nitrogen in the solution was determined by TOC/N analyzer. Soil-dissolved organic carbon and soil-dissolved organic carbon content per unit mass of dry soil were calculated. The available phosphorus content in the soil was measured by molybdate colorimetry [22]. Weigh 5 g air-dried soil into a 50 mL centrifuge tube, add 21 mL 0.5 M sodium bicarbonate solution and 1 g phosphorous-free activated carbon, shake for 40 min (200 r/min), filter, and take filtrate for use (soil extract). Add 5 g air-dried soil after screening into a 50 mL centrifuge tube, add 20 mL 0.5 M sodium bicarbonate solution, 1 g phosphate-free activated carbon and 1 mL 250 ug P/mL phosphorous labeled recovery solution, shock for 40 min (200 r/min), filter, and take filtrate for use. Absorb 1 mL of the above filter liquid into a 15 mL centrifuge tube, add 5 mL ultra-pure water and 0.2 mL dinitrophenol indicator in turn, add 1 mL 0.25M sulfuric acid until the yellow color just fades, then add 1mL molybdenum-antimony color developer, stand for 30 min, and determine the absorbance value at 712 nm wavelength. Calculating available phosphorus content in dry soil per unit mass. The carbon, nitrogen, and phosphorus contents of microbial biomass in the soil were determined using the chloroform fumigation extraction method [23, 24]. Following the protocol outlined by [25], the extracellular enzyme activity of soil microorganisms was determined [25]. The microbial extracellular enzymes tested included α-1,4-glucosidase and β-1,4-glucosidase (carbon acquisition enzymes), L-leucine aminopeptidase (a nitrogen acquisition enzyme), and alkaline phosphatase (a phosphorus acquisition enzyme). Buffer solutions were chosen based on the pH range of the soils under different treatments [44]. Except for L-leucine aminopeptidase, all reactions of soil microbial extracellular enzymes were incubated in the dark at 25ºC for 3 hours. Then, measurements were made using a multimode microplate reader at 355 and 460 nm wavelengths for excitation and emission, respectively.

Statistical Analysis

The stoichiometry of extracellular eco-enzymes was used to infer microbial metabolic limitations and carbon use efficiency in the soil. Microbial carbon limitation and microbial nutrient (nitrogen or phosphorus) limitation were represented by the calculation of vector length and angle, respectively, serving as indicators of soil microbial metabolic limitations. The computational methods for assessing soil microbial metabolic limitations and carbon use efficiency followed the protocol outlined by Cui et al. [26].

Both one-way and two-way analysis of variance were conducted to determine the statistical disparities in soil properties, microbial metabolic limitations, and carbon use efficiency. Fisher's least significant difference test was used to identify significant mean differences. Pearson correlation analyses were employed

to investigate the relationships between soil parameters and both microbial metabolic limitations and carbon use efficiency. Based on the outcomes of the Pearson correlation analyses, partial least squares path modeling was implemented to assess the potential pathway through which alterations in vegetation communities affect metabolic limitations and carbon use efficiency of soil microorganisms. All analyses were conducted using R software version 4.2.2 (R Core Team) [27]. Differences were considered significant at $p<0.05$.

Results and Discussion

The impact of Solidago Canadensis Invasion on soil EEA and Microbial Metabolic Limitation under Nitrogen Deposition Across Diverse Vegetation Communities

The average enzymatic activities for extracellular carbon (EEAC), nitrogen (EEAN), and phosphorus (EEAP) acquisition by microorganisms were found to range between 5.85-15.16, 4.82-13.60, and 3.02-28.47, respectively. Significant differences were observed across different treatments for EEAC, EEAN, and EEAP (all $p<0.01$). The multi-vegetation community treatment showed EEAC, EEAN, and EEAP values to be 0.79, 1.04, 0.91 times and 0.56, 1.26, 0.50 times greater than those of the *Artemisia argyi* and *Solidago decurrens* treatments respectively (Table 1). The respective ratios of EEAC to EEAN ($EEA_{C:N}$), EEAC to EEAP ($EEA_{C:P}$), and EEAN to EEAP ($EEA_{N:P}$) were 5.70-12.84, 1.29-2.28, and 1.00-3.23, each indicating a significant difference ($p<0.01$). However, under nitrogen deposition, variances were noted in the extracellular enzyme activity to stoichiometry ratio among different soils. The multi-vegetation community treatment displayed EEAC, EEAN, and EEAP values that were 1.00, 1.66, 0.10 times and 0.39, 1.45, 0.18 times those of the *Artemisia argyi* and *Solidago decurrens* treatments, respectively. As compared to the *Artemisia argyi* and *Solidago decurrens* treatments, the corresponding ratios of multi-vegetation for EEAC to EEAN ($EEA_{C:N}$), EEAC to EEAP ($EEA_{C:P}$), and EEAN to EEAP ($EEA_{N:P}$) were 8.04-27.51, 2.01-19.21, and 1.66-21.14, with each being significantly different (Table 1; $p<0.01$).

The findings indicated that: VL had significant positive correlations with NH_4-N ($R^2 = -0.74$, $p<0.01$), IN ($R^2 = -0.53$, $p<0.05$), MBN ($R^2 = 0.50$, $p<0.05$), $MB_{C:P}$ ($R^2 = 0.51$, $p<0.05$), $MB_{N:P}$ ($R^2 = 0.52$, $p<0.05$), EEAC ($R^2 = 0.74$, $p<0.01$), EEAN ($R^2 = -0.66$, $p<0.01$), $EEA_{C:N}$ ($R^2 = 0.99$, $p<0.01$), and $EEA_{N:P}$ ($R^2 = -0.71$, $p<0.01$). Similarly, VA demonstrated significant correlations with NH_4-N ($R^2 = -0.71$, $p<0.01$), MBN ($R^2 = 0.66$, $p<0.01$), EEAN ($R^2 = -0.77$, $p<0.01$), EEAP ($R^2 = 0.83$, $p<0.01$), $EEA_{C:N}$ ($R^2 = 0.71$, $p<0.01$), $EEA_{C:P}$ ($R^2 = -0.82$, $p<0.01$), and $EEA_{N:P}$ ($R^2 = -0.99$, $p<0.01$) (Fig. 2a). Thus, both VL and VA showed significant differences across different treatments (Fig. 1(e, f)). Random forest results showed that microbial extracellular enzyme activity and its stoichiometric ratio were the main influencing factors for VL and VA (Fig. 3(a, b)). Additionally, analysis via partial least squares path modeling suggested that alterations in the soil properties resulting from the invasion of *Solidago canadensis* in diverse vegetation communities influenced the soil microbial biomass. This affected extracellular enzyme activity and stoichiometric ratio, thereby influencing metabolic limitation (Fig. 3). Nevertheless, under the backdrop of nitrogen deposition, the pathway differed. Therefore, nitrogen deposition

Table 1. Soil extracellular enzyme and extracellular enzyme stoichiometric ratio among study sites, presented as means ± standard errors (n = 3).

Parameters	F	*p*	N_0			N_5		
			AS	A	S	AS	A	S
EEAC ($\times10^2$ nmol h^{-1} g^{-1} soil)	18.94	**	7.46±1.55bc	9.45±0.48b	13.33±1.09a	5.85±0.85c	5.85±0.57c	15.16±0.28a
EEAN (nmol h^{-1} g^{-1} soil)	44.80	**	13.06±0.33a	13.18±0.04a	10.36±0.26b	7.99±1.18c	4.82±0.40d	5.52±0.19d
EEAP ($\times10^2$ nmol h^{-1} g^{-1} soil)	236.15	**	5.19±0.05d	4.39±0.80de	10.35±0.16c	3.05±0.02e	29.09±0.36a	16.61±1.32b
$EEA_{C:N}$ ($\times10^2$)	42.57	**	0.57±0.11c	0.72±0.04c	1.28±0.08b	0.80±0.25c	1.22±0.08b	2.75±0.5a
$EEA_{C:P}$	10.74	**	1.43±0.29bc	2.28±0.34a	1.29±0.12bc	1.92±0.28ab	0.20±0.02d	0.92±0.07c
$EEA_{N:P}$ ($\times10^{-2}$)	18.91	**	2.52±0.04a	3.23±0.63a	1.00±0.04b	2.61±0.37a	0.17±0.01b	0.34±0.03b

EEAC = soil extracellular C-acquisition enzymes; EEAN = soil extracellular N-acquisition enzymes; EEAP = soil extracellular P-acquisition enzymes; $EEA_{C:N}$ = the ratio of soil extracellular C-acquisition enzymes to N-acquisition enzymes; $EEA_{C:P}$ = the ratio of soil extracellular C-acquisition enzymes to P-acquisition enzymes; $EEA_{N:P}$ = the ratio of soil extracellular N-acquisition enzymes to P-acquisition enzymes. * = significant at the level of $p<0.050$, and ** = significant at the level of $p<0.010$; ns = not significant at the level of $p>0.05$. Different lower-case letters above column represent significant differences among the study sites. The single vegetation community consisted of either four *Artemisia argyi* (A) or four *Solidago decurrens* (S), while the multiple vegetation community consisted of a mix of two *Artemisia argyi* and two *Solidago decurrens* (AS).

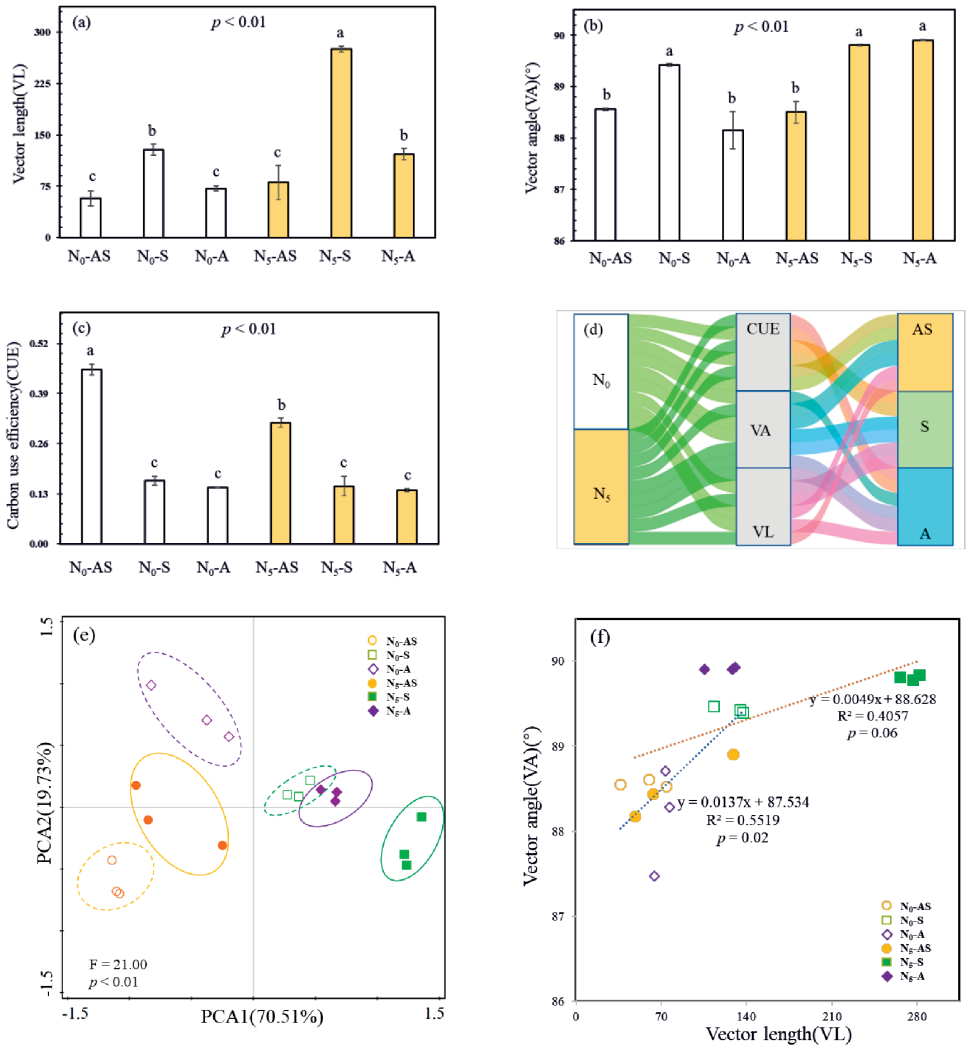

Fig. 1. Changes of soil microbial metabolic limitation and carbon use efficiency under the background of different vegetation communities and nitrogen deposition. a) Microbial carbon limitation (VL); b) Microbial nitrogen and phosphorus limitation (VA); c) Microbial carbon use efficiency (CUE); d) Sankey diagram of VL, VA and CUE to nitrogen deposition and vegetation community; e) PCA (principal component analysis) of VL, VA and CUE; and f) Scatter diagram for VA and VL. Different lower-case letters above column represent significant differences (p<0.050) among the study sites. The single vegetation community consisted of either four *Artemisia argyi* (A) or four *Solidago decurrens* (S), while the multiple vegetation community consisted of a mix of two *Artemisia argyi* and two *Solidago decurrens* (AS).

altered soil nutrients, which affected soil microbial biomass, ultimately impacting metabolic limitations. The effect of extracellular enzymes on VL and VA was positive, while the impact of the extracellular enzyme stoichiometric ratio on VL and VA was negative.

The pattern of metabolic limitation in soil microorganisms, as indicated by the stoichiometry of extracellular ecological enzymes, varies under different treatments, with vector length (VL) and angle (VA) values ranging from 56.98-275.15 and 88.15-89.91°, respectively. The VL in multiple vegetation communities is significantly lower than that in single *Artemisia argyi* and *Solidago decurrens* communities, exhibiting a reduction of 20.59% and 55.64%, respectively (Fig. 1a; p<0.01). However, the overall trend of soil microbial carbon limitation in various vegetation communities remains consistent under the nitrogen deposition treatment, but all display an increase compared to treatments without nitrogen deposition. With the backdrop of nitrogen deposition, the multi-vegetation community has significantly lower levels than the single *Artemisia argyi* vegetation community and *Solidago decurrens*, demonstrating a reduction by 55.70% and 70.75%, respectively. The trend of VA changes under different treatments mirrors VL (with the exception of *Solidago decurrens* treatment without nitrogen deposition). The VA in the multiple vegetation community treatments is significantly lower than that in single *Solidago decurrens* (55.64%) (Fig. 1b; p<0.01). Compared to treatments without nitrogen deposition, nitrogen limitation in soil microorganisms across various vegetation communities

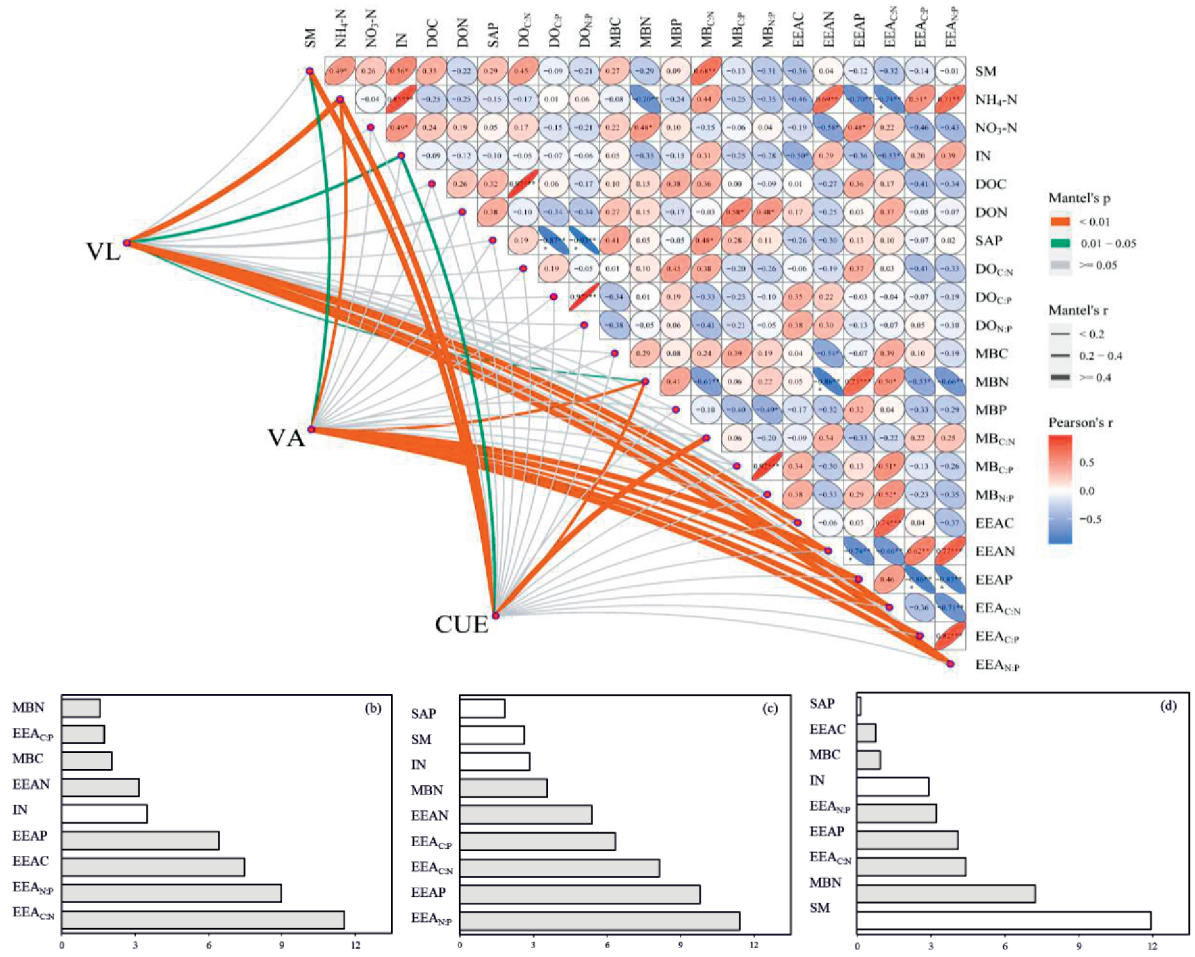

Fig. 2. Mantel test and random forest of the results between the soil parameters and microbial metabolic limitations and carbon use efficiency. a) Result of mantel test between the soil parameters and microbial metabolic limitations and carbon use efficiency; b) Result of microbial carbon limitation (VL); c) Result of microbial nitrogen and phosphorus limitation (VA); d) Result of microbial carbon use efficiency (CUE). VL = vector length; VA = vector angle; CUE = microbial carbon use efficiency; SM = soil moisture; NH_4-N = ammonia nitrogen; NO_3-N = nitrate nitrogen; IN = inorganic nitrogen; EEAC = carbon-acquisition microbial extracellular enzyme activity; DOC = dissolved organic carbon; DON = dissolved organic nitrogen; SAP = available phosphorus; $DO_{C:N}$ = ratio of DOC to DON; $DO_{C:P}$ = ratio of DOC to SAP; $DO_{N:P}$ = ratio of DON to SAP; MBC = microbial biomass carbon; MBN = microbial biomass nitrogen; MBP = microbial biomass phosphorus; $MB_{C:N}$ = ratio of MBC to MBN; $MB_{C:P}$ = ratio of MBC to MBP; $MB_{N:P}$ = ratio of MBN to MBP; EEAN = nitrogen-acquisition microbial extracellular enzyme activity; EEAP = phosphorus-acquisition microbial extracellular enzyme activity; $EEA_{C:N}$ = the ratio of EEA_C to EEA_N; $EEA_{C:P}$ = the ratio of EEA_C to EEA_P; $EEA_{N:P}$ = the ratio of EEA_N to EEA_P. *** = significant at the level of $p<0.001$, ** = significant at the level of $p<0.010$, and * = significant at the level of $p<0.050$.

has been mitigated under the nitrogen deposition treatment, while phosphorus limitation has escalated. The VA of multiple vegetation communities is notably lower than that of single *Artemisia argyi* vegetation community and *Solidago decurrens*, demonstrating a decrease by 1.56% and 1.46%, respectively. Additionally, all VA values are greater than 45 degrees, which shows phosphorus limitation in the microbial communities across all treatments. As a result, phosphorus turns out to be the ingredient that limits soil microbe growth at all sites.

Invasive alien plants, such as *Solidago canadensis*, tend to grow rapidly, thereby achieving dominance in the ecological environment by swiftly absorbing and utilizing soil nutrients compared to the existing vegetation and soil microbes [5, 26]. Consequently, the invasion of *Solidago canadensis* could intensify the nutrient limitations and imbalances concerning soil microbes, potentially having further direct and indirect detrimental effects on microbial resource acquisition and thereby impeding the growth and development of soil microbes [27-29]. During the *Solidago canadensis* invasion, the plant-derived inputs into the soil (primarily root exudates) may serve as an important mechanism regulating soil nutrient cycling. Past studies have shown that interactions with substrates produced by *Solidago canadensis* can decrease the activity of the enzymes responsible for acquiring carbon, nitrogen, and phosphorus [30]. Accordingly, it is possible that the interactions between substances released by plant allelopathy, secondary chemicals in the soil, and microbial functional groups

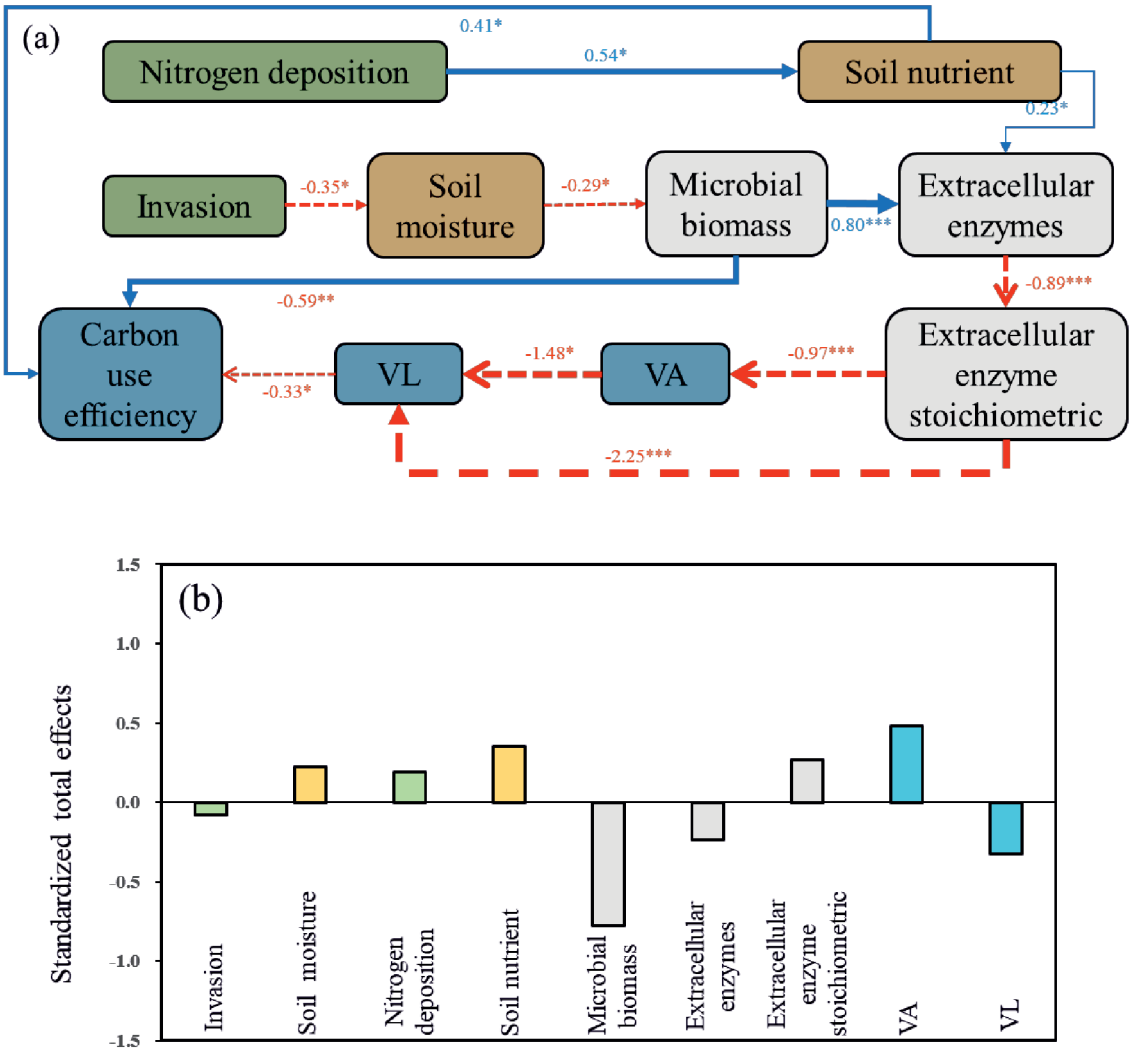

Fig. 3. Partial least squares path modeling result shows potential influence paths of vegetation community alteration on soil microbial metabolic limitations and carbon use efficiency. Blue and red arrows indicate positive and negative flows of causality. *** = significant at the level of $p<0.001$; ** = significant at the level of $p<0.010$ and * = significant at the level of $p<0.050$. VL = microbial carbon limitation; VA = microbial nitrogen and phosphorus limitation.

are responsible for the changes in enzyme activity seen among various vegetation communities during invasion [30, 31].

Therefore, we observed a significant discrepancy in microbial carbon limitation between the single vegetation community invaded by *Solidago canadensis* and the multi-vegetation community ($p<0.01$), highlighting the carbon nutrient limitation in the microbial metabolism across different *Solidago canadensis* vegetation communities (Fig. 1a) which is in line with our first hypothesis. The minimum microbial carbon limitation in the multi-plant community treatment indicates that vegetation community diversity may act as a buffer against the impacts of *Solidago canadensis* invasion on the carbon limitation of soil microorganisms in invaded areas. Single-species ecosystems with low diversity show lesser resistance to the invasion of *Solidago canadensis*, leading to most nutrients being consumed by it, thereby causing soil microbes to face a carbon shortage from the environment, eventually reflecting as intensified carbon limitation. However, the complex ecosystem composition of the multi-vegetation community treatment offers stronger resistance against invasive plants, propelling it to compete with invasive plants for essential nutrients, thereby reducing carbon limitation in multi-vegetation community areas [32, 33]. In essence, invaded regions with high diversity demonstrate greater resilience than those with low diversity.

While nitrogen limitation is reduced under the influence of nitrogen deposition, microbial carbon and phosphorus limitations are increased. This may be because the soil ecosystem is trying to maintain element homeostasis, which encourages soil microbes to increase their demand for carbon and phosphorus, which ultimately results in an increase in carbon and

phosphorus limitations [34]. Moreover, all VA points exceed the 1:1 line (VA greater than 45°), indicating that phosphorus is the limiting factor for soil microbes in all treatments which supports our second hypothesis. Elevated phosphorus limitation can significantly impact microbial growth and metabolism, as microbes need to maintain adequate nutrients for growth and development, and a high phosphorus limitation environment favors the successful invasion of *Solidago canadensis* [34]. Hence, the exacerbation of soil microbial phosphorus limitation under the influence of nitrogen deposition is more likely to result in a successful *Solidago canadensis* invasion. Still, the metabolic limitation of the multi-vegetation community under nitrogen deposition remains weak, indirectly reflecting the community's resilience against various environmental stressors. Furthermore, the study by [35] demonstrates that the interaction and complementary effects among microbes can mitigate metabolic limitations in stressful environments, thereby fostering more efficient community growth [35]. The interspecies complementing effects of multi-vegetation community treatments in the microbial community will be more pronounced, lowering metabolic limitation, as habitats with high vegetation community diversity frequently harbour a more abundant microbial community.

The competition for soil nutrients between invasive and local plants, as well as between plants and soil microorganisms, could precipitate nutrient limitation and imbalance [36]. Concurrently, we postulate that the invasion of *Solidago canadensis* can alter soil moisture and nutrient inputs, thereby affecting nutrient availability. This hypothesis is supported by the findings of our random forest and PLS-PM analyses. Nutrient limitation and imbalance further curtail enzymatic activity (Figs 3, 4), as soil microorganisms can modulate enzyme production and the stoichiometry of ecological enzymes, especially in nutrient-deficient microenvironments [13]. Soil moisture impacts enzymes by influencing microbial biomass, leading to relative microbial carbon and/or phosphorus limitation (Fig. 3). Consequently, soil microorganisms may strive to enhance the acquisition of limiting carbon and phosphorus to maintain stoichiometric homeostasis and sustain growth under nutrient-scarce conditions [36, 37], a response that might be triggered by the invasion of *Solidago canadensis*. Under the nitrogen deposition treatment, the further intensification of carbon-phosphorus limitation implies that nitrogen deposition and *Solidago canadensis* invasion collectively constrain soil microbial metabolism and carbon utilization efficiency via synergistic mechanisms. In summary, the invasion of *Solidago canadensis*, in the context of nitrogen deposition, significantly influences the metabolic limitation of soil microorganisms. However, multi-vegetation communities demonstrate greater resilience in this process compared to single-vegetation communities.

The Impact of Solidago Canadensis Invasion on Soil Microbial Carbon Use Efficiency under Nitrogen Deposition Across Diverse Vegetation Communities

Microbial carbon use efficiency was significantly correlated with SM (R^2 = 0.77, p<0.01), NH_4-N (R^2 = 0.72, p<0.01), IN (R^2 = 0.91, p<0.05), MBN (R^2 = -0.69, p<0.01), $MB_{C:N}$ (R^2 = 0.84, p<0.01), EEAP (R^2 = -0.53, p<0.05), $EEA_{C:N}$ (R^2 = -0.49, p<0.05) and $EEA_{N:P}$ (R^2 = 0.87, p<0.01) (Fig. 2a). The carbon use efficiency of soil microbes across different treatments varies from 13.92% to 45.20%, and the differences are significant (p<0.05). Relative to the carbon use efficiency of soil microbes in the multi-vegetation community treatment, single *Artemisia argyi* and *Solidago canadensis* vegetation communities exhibited a decrease of 63.78% and 67.72%, respectively (Fig. 1c). However, under the nitrogen deposition treatment, the overall trend of soil microbial carbon use efficiency among various vegetation communities remained unaltered, although all exhibited a decrease relative to the treatment without nitrogen deposition. The multi-vegetation community, single *Artemisia argyi* vegetation community, and single *Solidago decurrens* vegetation community showed a decrease of 30.31%, 4.59%, and 8.49%, respectively. A significant positive correlation was observed between the carbon use efficiency of soil microbes and VL (R^2 = 0.85, p<0.01; Fig. 2). Random forest results showed that microbial biomass and soil moisture were the main influencing factor concerning carbon use efficiency (Fig. 3c). Furthermore, the partial least squares path model indicates that soil moisture influences the carbon use efficiency not only by impacting microbial biomass but also by altering the activity and stoichiometric balance of extracellular enzymes. In contrast, nitrogen deposition affects the activity of microbial extracellular enzymes, thereby influencing the carbon use efficiency of microorganisms, using soil nutrients as a driving factor (Fig. 3). In addition, soil nutrients, microbial biomass and VL were identified as the direct driving factors for alterations in the microbial carbon use efficiency across all treatments.

Our study observed considerable variations in soil microbial carbon use efficiency across different treatments. The efficiency in multi-vegetation community treatments was notably higher than that in single vegetation community treatments. In contrast, no significant difference was observed between the *Artemisia argyi* vegetation community and *Solidago decurrens* vegetation community treatments (Fig. 1c). Microbial carbon use efficiency quantifies the proportion of carbon dedicated to microbial growth absorption from the total carbon sources [15]. A significant increase in microbial carbon use efficiency recorded in treatments characterized by high vegetation community diversity implies that microbial respiration consumes less carbon, leading to a greater amount of fixed carbon in microbial biomass. This further underscores the crucial role of the

ecosystems with high vegetation community diversity which plays a greater role in the global ecosystem carbon cycling [16]. However, nitrogen deposition treatment inhibited the microbial carbon use efficiency across all three vegetation communities, highlighting a tight interconnection between carbon cycling and nitrogen deposition.

Plant matrix inputs into the soil (predominantly via root secretions) might represent another significant mechanism accounting for the disparities in the soil microbial carbon use efficiency among distinct vegetation communities. The allelochemicals present in plant matrix inputs directly influence microbial carbon absorption and utilization [6]. As a result, *Solidago canadensis* invasion stimulates competition among microbial populations as well as competitiveness for resources between plants and microorganisms. This competitive dynamic may further modify the physiology and behaviour of individual microbes, alter the structure and function of microbial communities, and ultimately affect the carbon use efficiency of microbes [38]. Furthermore, increased moisture promotes the growth of *Solidago canadensis*, which favours moist environments [39]. Thus, to counterbalance microbial carbon limitation and mitigate the detrimental effects of changes in water and nutrients prompted by *Solidago canadensis* invasion, microbes make trade-offs. They allocate less energy towards new biomass synthesis and more energy towards associated extracellular enzymes [16]. This ultimately results in lower carbon use efficiency in single-vegetation community treatments compared to multi-vegetation community treatments, which supports our first hypothesis.

Furthermore, the partial least squares path model suggests that soil moisture can impact microbial carbon use efficiency not only by affecting microbial biomass but also by modulating the activity and stoichiometric balance of extracellular enzymes (Fig. 3). These findings proposed that soil moisture is a pivotal factor that influences soil microbial carbon use efficiency by transforming the structure and function of microbial communities [12], supporting the third hypothesis. The strong correlation between soil moisture, microbial carbon limitation, and carbon use efficiency aligns with prior studies. These studies asserted that changes in soil moisture could directly or indirectly modify the composition, structure, and function of microbial communities, thereby indirectly impacting microbial carbon use efficiency [12]. Previous research suggests that microbial carbon use efficiency declines with prolonged drought [40]. Additionally, limited water availability restricts the substrate supply for microbial cells, resulting in a larger proportion of substrate being allocated to maintain metabolism and a smaller proportion available for growth. Consequently, water limitation is anticipated to reduce microbial carbon use efficiency [41]. However, during the invasion of *Solidago canadensis*, the pattern of changes in microbial carbon use efficiency is somewhat adverse to the pattern of microbial carbon limitation, that is, low carbon limitation correlates with high carbon use efficiency, and high carbon limitation associates with low carbon use efficiency (Figs 1, 4). The reduction in microbial carbon limitation induced by soil moisture in the multi-vegetation community treatment led to a corresponding augmentation in carbon use efficiency. This is due to the trade-off between microbial carbon limitation and carbon usage efficiency, wherein bacteria utilize their growth and development instead of the energy needed to produce hydrolytic enzymes [41], further demonstrating the superior tolerance of multi-vegetation communities to *Solidago canadensis* invasion. Under the nitrogen deposition backdrop, the soil microbial carbon use efficiency of all treatments diminishes, indicating that alterations in soil nutrients instigated by nitrogen deposition may disrupt the original soil nutrient equilibrium, somewhat alleviate microbial nitrogen limitation (Table S1), and amplify microbial carbon demand due to excessive nitrogen nutrients [36-37]. This, in turn, leads to an increase in microbial carbon limitation and a decrease in carbon use efficiency. The PLS-PM results support the second hypothesis of our results.

Implication

Changes in vegetation communities, a vital component of ecosystems around the globe, have the power to alter how the hydrological system works. These alterations could influence the composition, structure, and functioning of soil microbial communities, thereby modifying the carbon cycling process within soil ecosystems [42, 43]. Previous research has typically examined singular scenarios – such as the impact of invasive alien species or nitrogen deposition on soil microbial carbon use efficiency. However, given the complexity of ecosystem influencers, examining a single factor no longer holds practical relevance. Thus, this study examines the influence of *Solidago canadensis* invasion in the context of nitrogen deposition on the metabolism and carbon use efficiency of soil microbes within local vegetation communities. This offers theoretical guidance for future research on soil ecosystem carbon cycling. In this investigation, nitrogen deposition, serving as a nutrient input, altered the initial impact of *Solidago canadensis* on the carbon use efficiency of soil microbes across different vegetation communities within the invaded area. Nevertheless, the general direction of the impact remained consistent. Hence, the invasion of *Solidago canadensis* combined with nitrogen deposition may jointly affect soil ecosystem carbon cycling, demanding further research.

Conclusion

The invasion of *Solidago canadensis*, facilitated by soil moisture, induces alterations in the extracellular

enzyme activity and its stoichiometric ratio within soil microbes of the invaded area, subsequently influencing microbial metabolism and carbon use efficiency. These findings indicate that multi-vegetation communities exhibit greater resilience against *Solidago canadensis* invasion compared to single-vegetation communities. As a result, microbial carbon limitation within multi-vegetation community treatments is less severe, leading to enhanced carbon use efficiency. A positive correlation is observed between microbial carbon use efficiency and carbon limitation, and a high correlation exists with the soil moisture contents. In conjunction with *Solidago canadensis*, nitrogen deposition, which is encouraged by soil nutrients, affects the efficiency of soil microbial carbon utilization. This investigation provides an initial conclusion regarding the shifts in metabolic limitations and carbon use efficiency of soil microbes across different vegetation communities. These insights may facilitate the exploration of the feedback and response of carbon cycling in diverse vegetation community ecosystems under the combined influence of nitrogen deposition and invasions.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Material

Table S1. Soil properties among study sites, presented as means ± standard errors (n = 3).

Parameters	F	*p*	N_0			N_5		
			AS	A	S	AS	A	S
SM (×10 %)	31.71	**	2.71±0.07a	1.63±0.10e	2.23±0.02c	2.19±0.08b	2.21±0.07c	2.07±0.08d
NH_4-N (μg g^{-1} soil)	11.60	**	2.07±0.22a	1.45±0.06b	1.17±0.05bc	1.70±0.12ab	0.74±0.34cd	0.48±0.02d
NO_3-N (×10^{-1} μg g^{-1} soil)	2.14	ns	4.59±1.27	2.45±0.95	3.50±0.35	7.52±2.23	9.67±3.15	6.93±1.82
IN (μg g^{-1} soil)	2.48	ns	2.53±0.33	1.69±0.11	1.52±0.03	2.45±0.34	1.70±0.65	1.17±0.17
DOC (×10^{-1} mg g^{-1} soil)	1.44	ns	1.93±0.15	1.62±0.17	1.86±0.05	1.82±0.08	1.97±0.02	1.90±0.05
DON (×10^{-2} mg g^{-1} soil)	1.93	ns	3.72±0.06	3.78±0.12	3.66±0.04	3.87±0.07	3.74±0.03	3.95±0.11
SAP (×10^{-1} mg g^{-1} soil)	3.19	*	3.09±0.63a	2.15±0.13b	1.37±0.04b	3.18±0.43a	2.97±0.54a	3.06±0.32a
$D_{OC:}N$	2.39	ns	5.20±0.38	4.27±0.36	5.08±0.11	4.70±0.17	5.26±0.09	4.80±0.16
$DO_{C:P}$	10.38	**	0.66±0.10b	0.76±0.09b	1.36±0.08a	0.60±0.09b	0.70±0.11b	0.63±0.06b
$DO_{N:P}$ (×10^{-1})	10.88	**	1.30±0.23b	1.77±0.15b	2.67±0.10a	1.26±0.16b	1.34±0.22b	1.31±0.11b
MBC (×10^{-1} mg g^{-1} soil)	4.08	*	3.6±1.08bc	0.91±0.04c	3.12±0.45bc	9.65±2.93a	3.71±0.27bc	7.35±2.20ab
MBN (×10^{-2} mg g^{-1} soil)	26.50	**	0.16±0.01e	0.83±0.04d	1.16±0.26cd	1.29±0.09bc	2.02±0.06a	1.55±0.11b
MBP (×10^{-2} mg g^{-1} soil)	0.52	ns	2.49±0.97	1.46±0.25	2.65±1.89	2.62±0.36	4.22±1.75	2.54±1.12
$MB_{C:N}$ (×10)	6.85	**	22.80±7.19a	1.10±0.08b	2.79±0.21b	7.34±2.15b	1.85±0.19b	4.74±1.39b
$MB_{C:P}$ (×10)	0.96	ns	2.34±1.16	0.65±0.09	2.65±1.05	3.50±0.64	1.48±0.76	11.21±9.43
$MB_{N:P}$ (×10^{-1})	0.89	ns	1.01±0.52	6.10±1.32	8.98±3.26	5.09±0.55	8.40±4.77	18.56±14.08

SM = soil moisture; NH_4-N = ammonia nitrogen; NO_3-N = nitrate nitrogen; IN = inorganic nitrogen; DOC = dissolved organic carbon; DON = dissolved organic nitrogen; SAP = available phosphorus; $DO_{C:N}$ = ratio of DOC to DON; $DO_{C:P}$ = ratio of DOC to SAP; $DO_{N:P}$ = ratio of DON to SAP; MBC = microbial biomass carbon; MBN = microbial biomass nitrogen; MBP = microbial biomass phosphorus; $MB_{C:N}$ = ratio of MBC to MBN; $MB_{C:P}$ = ratio of MBC to MBP; $MB_{N:P}$ = ratio of MBN to MBP. * = significant at the level of $p<0.050$, and ** = significant at the level of $p<0.010$; ns = not significant at the level of $p>0.05$. Different lower-case letters above column represent significant differences among the study sites. The single vegetation community consisted of either four *Artemisia argyi* (A) or four *Solidago decurrens* (S), while the multiple vegetation community consisted of a mix of two *Artemisia argyi* and two *Solidago decurrens* (AS).