agronomy

MDPI

Article

Responses of Soil Enzyme Activity to Long-Term Nitrogen Enrichment and Water Addition in a Typical Steppe

Jinbao Zhang [1,2], Ke Jin [2], Yonghong Luo [1], Lan Du [1], Ru Tian [1], Shan Wang [1], Yan Shen [1], Jiatao Zhang [1], Na Li [1], Wenqian Shao [1] and Zhuwen Xu [1,*]

[1] School of Ecology and Environment, Inner Mongolia University, Hohhot 010021, China; 21915012@mail.imu.edu.cn (J.Z.); 22215011@mail.imu.edu.cn (L.D.); 32015019@mail.imu.edu.cn (R.T.); 32015021@mail.imu.edu.cn (S.W.); 22015013@mail.imu.edu.cn (Y.S.); 22115009@mail.imu.edu.cn (J.Z.); 32015206@mail.imu.edu.cn (N.L.); 32115006@mail.imu.edu.cn (W.S.)

[2] Grassland Research Institute, Chinese Academy of Agricultural Sciences, Hohhot 010010, China; jinke@caas.cn

* Correspondence: zwxu@imu.edu.cn

Abstract: Enzyme activity plays an important role in soil biochemical processes and is a key factor driving nutrient cycling. Although a great number of studies examined the effects of nitrogen (N) enrichment and water (W) addition on soil enzyme activity, most of them focused on the effect of only one resource and are based on short-term investigations. The separate and interactive effects of long-term changes in nitrogen and water on soil enzyme activity remain largely unexplored. In this study, we demonstrated the responses of two types of soil enzyme, β-1,4-glucosidase (BG) and acid phosphatase (APA), to increased nitrogen and water based on a 16-year experiment conducted in a typical grassland in northern China. The results show that: (1) nitrogen addition inhibited BG and APA in 2019 and 2020; (2) water addition had no significant effect on BG activity, but significantly reduced APA activity in 2020; and (3) redundancy analysis (RDA) showed that nitrogen and water addition affected soil enzyme activity mainly by affecting soil microbial biomass carbon (MBC). The present research offers a comprehensive explanation of how atmospheric nitrogen deposition and precipitation patterns affect the characteristics of microorganisms and the cycling of nutrients in grassland ecosystems.

Keywords: acid phosphatase; β-1,4-glucosidase; climate change; temperate grassland; nitrogen deposition; precipitation

check for updates

Citation: Zhang, J.; Jin, K.; Luo, Y.; Du, L.; Tian, R.; Wang, S.; Shen, Y.; Zhang, J.; Li, N.; Shao, W.; et al. Responses of Soil Enzyme Activity to Long-Term Nitrogen Enrichment and Water Addition in a Typical Steppe. *Agronomy* **2023**, *13*, 1920. https://doi.org/10.3390/agronomy13071920

Academic Editor: Kesi Liu

Received: 13 June 2023
Revised: 12 July 2023
Accepted: 17 July 2023
Published: 20 July 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Global increase in nitrogen deposition [1] and changes in precipitation pattern [2] fundamentally changed the physicochemical properties and biochemical cycling of soils [3–5]. Soil microbes are key drivers of nutrient cycling, and soil enzyme regulation is the most important process in microbial regulation of nutrient cycling [6]. To meet microbial growth under conditions of soil resource scarcity, soil microorganisms secrete enzymes [7]. Enzymes, as product of soil biological activity, are indicator of microbial nutrient requirements. Soil enzyme activity reflects the rate of soil microbial metabolism and biochemical cycling processes. The soil enzyme converts complex organic matter into plant-available nutrients and is a key factor in determining soil organic matter decomposition and nutrient cycling [8–10]. β-1,4-glucosidase (BG) is a soil enzyme secreted by cellulose, which often takes soil carbon as the matrix and participates in the carbon cycle. Phosphorus transformation-related acid phosphatase (APA) hydrolyzes the ester bonds in soil organic phosphorus to promote phosphorus conversion, determining the phosphorus availability for plants. Soil enzyme activity would change with environmental fluctuations. While there is a growing amount of research investigating the effects of changes in precipitation or nitrogen availability on soil enzyme activity, the majority of these studies focused

primarily on the individual effects of nitrogen or water [11]. Unexpectedly, only a limited number of studies investigated how soil enzyme activity responds to simultaneous changes in these two major drivers of global change [12–14]. Experimental studies explored the effect of nitrogen addition on microbial enzyme activity and found controversial results, e.g., some studies suggested that nitrogen addition would induce soil acidification and inhibits enzyme activity [15], and some found nitrogen addition stimulates or does not affect soil enzyme activity [13,16–19]. Similarly, previous studies also found mixed effects of increased precipitation on soil enzyme activity. For example, increased precipitation was shown to increase soil enzyme activity by promoting substrate diffusion [20]. Nonetheless, the introduction of rainfall may also impede the functioning of enzymes responsible for carbon (C) and phosphorus (P) absorption due to its impact on the physical and chemical characteristics of the soil [9]. The insignificant effect of water addition on soil enzyme activity was also reported in grassland ecosystems [16]. Furthermore, it was demonstrated that water addition could mitigate the effects of nitrogen enrichment on microorganisms by leaching or reducing the accumulation of inorganic nitrogen [21,22] and produce a significant effect on soil enzyme activity. Thus, it is necessary to explore their combined effects to understand and predict how soil enzyme activity will respond to climate change. Furthermore, most of the relevant studies are based on short-term investigation; we still lack a profound understanding of the effects of long-term changes in nitrogen and water on soil enzyme activity in arid and semi-arid grassland ecosystems.

Elemental stoichiometry and soil nutrient content were reported as key factors influencing soil enzyme activity [23–27]. For example, P-acquisition enzymes are stimulated at certain concentrations of nitrogen [27–29]. At the same time as nitrogen utilization increasing, carbon acquisition enzyme activity shows either a positive response [23] or no changes [19]. A study at 17 sites in the USA found that the most important regulator of soil enzyme activity was soil C and N concentrations. Nitrogen enrichment and water addition would change soil nutrients and inorganic nitrogen components [9,11,20,21] and microbial communities [30], and then affect soil enzyme activity.

The temperate grassland in northern China constitutes an important part of the Eurasian grassland [3] and plays a key role in servicing the ecological environment and socio-economics of the region [31]. Summer precipitation [32] and nitrogen deposition [33,34] are both expected to increase in this grassland area. These global change drivers fundamentally change the grassland ecosystem, and the intermediate changes are largely regulated by micro-biological processes, such as soil enzyme activities. However, the specific patterns and the underlying mechanisms of these processes remain largely unknown. In this study, the effects of nitrogen and water additions on the activities of BG and APA were investigated based on a 16-year field manipulative experiment (since 2005). Our aim is to reveal the individual and interactive effects of long-term increase in nitrogen deposition and precipitation on the soil enzyme that is related to C and P nutrient cycling.

2. Materials and Methods

2.1. Study Sites and Experimental Design

The study site is situated in Duolun County, Inner Mongolia, China. Figure 1 shows the elevation of 1324 m above sea level at the coordinates (116°17′ E and 42°02′ N). The climate in the area is marked by semi-arid continental monsoon rainfall, meaning the spring season is characterized by dry weather, while the summer season is known for its wet conditions. Climate: 2.1 °C, precipitation: 379 mm, soil: chestnut calcium [35]. The study area is primarily characterized by the prevalence of *Stipa krylovii*, *Agropyron cristatum*, and *Artemisia frigida* within its plant communities. Seven blocks of naturally formed communities were established using the split-plot experimental design in early April 2005. Each block received treatments of natural rainfall and supplemental water. The main plot treatment was precipitation, while subplot treatment was nitrogen. The N treatment (ambient N or N addition) was randomly assigned to each main plot, which was divided into two subplots measuring 8 m × 8 m. During the months of June to

August, the irrigated areas received a weekly application of 15 mm of water, resulting in a cumulative supply of 180 mm throughout the growing period. Urea was used to supply 10 g N $year^{-1}$ m^{-2} to the nitrogen addition plots, with equal amounts provided in early May and late June. In this study, four different treatments were employed: control (without nitrogen and water addition), nitrogen addition, water addition, and nitrogen combined with water condition.

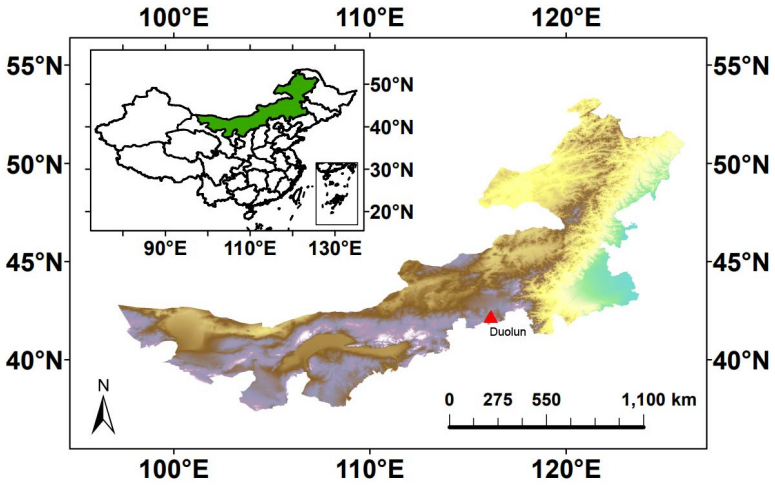


Figure 1. Location of the study site.

2.2. Soil Sampling and Chemical Property Analysis

From May to September in 2019, soil samples were collected every two weeks in each subplot. A total of five soil cores, measuring 3 cm in diameter and 10 cm in depth, were obtained. The percentage weight loss of fresh soil cores was determined by calculating the soil moisture as the difference in weight between fresh and dry soil cores after they were weighed and dried at a temperature of 105 °C for a duration of 48 h. At the conclusion of August in the years 2019 and 2020, seven soil samples were randomly chosen from every plot. The levels of inorganic nitrogen in soil samples were measured with a FIAstar 5000 Analyzer, a flow injection autoanalyzer manufactured by Foss Tecator in Denmark. We stirred dry soil in CO_2-free deionized water (1:2.5 soil to water ratio) for one minute and allowed the soil to stabilize for an hour before measuring its pH using a pH meter (METTLER TOLEDO, FE28, Zurich, Switzerland).

2.3. Soil Enzyme Activities Analysis

The activities of BG and APA were analyzed according to Treseder [36]. For this test, a fresh soil sample weighing 1 g was combined with 4 milliliters of adjusted universal buffer (pHBG = 6.0, pHacid APA = 6.5) and 1 milliliter of substrate (pHAPS = 6.5). BG is represented by p-nitrophenol-β-D-glucopyranoside, while APA is represented by p-nitrophenyl-phosphate. After the reaction, 4 mL of Tris (hydroxymethyl) aminomethane was introduced with a concentration of 0.1 M and 1 mL of $CaCl_2$ (THAM, pH = 12) at 37 °C. The reaction in the case of APA was halted by the addition of 1 mL of $CaCl_2$ and 4 mL of NaOH. Following the filtration process, the solution was assessed at a wavelength of 450 nm using a UV-VIS spectrometer treasurer hectometer (MAPADA, P9, Shanghai, China). The activities of BG and APA were measured in milligrams of p-nitrophenol (PNP) per kilogram per hour.

2.4. Statistical Analysis

ANOVAs with split plot designs were employed to examine the impact of water on soil enzymatic activity, specifically focusing on the interaction between nitrogen and water. A study was performed to analyze the correlation between soil enzymatic activity and soil moisture (SM) using Pearson correlation analysis. The study also considered soil

temperature (ST), pH, total carbon (TC), total nitrogen (TN), total phosphorus (TP), available phosphorus (AP), inorganic nitrogen (IN), nitrate nitrogen (NO_3^-−N), ammonium nitrogen (NH_4^+−N), TC:TN, TN:TP, soil microbial biomass carbon (MBC), soil microbial biomass nitrogen (MBN), and MBC:MBN. All statistical analyses were performed using the R software (V.3.3.2), and $p < 0.05$ was considered a statistically significant difference. Redundancy analysis (RDA) was undertaken using the Canoco software (Canoco for Windows 5.02). All bar graphs were drawn using Origin 2021 (https://www.originlab.com/, accessed on 1 May 2023).

3. Results

3.1. Effects of Nitrogen and Water Addition on the Physical and Chemical Properties of Soils

Soil temperature experienced significant alterations as a result of both nitrogen enrichment and the addition of water ($p < 0.001$, Table 1, Figure 2A). The addition of water significantly affected soil moisture, while the addition of nitrogen had no effect on SM (Table 1). Water increased SM by 71.57% ($p < 0.05$, Figure 2B), the simultaneous addition of nitrogen and water increased SM by 82.81% ($p < 0.05$). In 2019, adding water increased SM by 84.32% ($p < 0.05$), while adding nitrogen and water simultaneously increased SM by 87.15% ($p < 0.05$), and adding nitrogen had no effect. In 2020, both nitrogen and water increased SM, nitrogen application increased SM by 20.03% ($p < 0.05$), and water addition increased SM by 56.01% ($p < 0.05$), the simultaneous addition of nitrogen and water increased SM by 77.53 ($p < 0.05$). Both nitrogen and water had an effect on pH ($p < 0.001$, Table 1), and the simultaneous addition of nitrogen and water had an interactive effect on pH (p <0.01, Table 1). Addition of nitrogen reduced pH by 14.57% ($p < 0.05$, Figure 2C), addition of water increased pH by 8.65% (p <0.05), and simultaneous addition of nitrogen and water reduced pH by 4.10% ($p < 0.05$). In 2019, addition of nitrogen decreased pH by 22.14% (p <0.05), addition of water increased pH by 3.72% ($p < 0.05$), and simultaneous addition of nitrogen and water decreased pH by 10.67% ($p < 0.05$). In 2020, addition of nitrogen reduced pH by 15.74% ($p < 0.05$), while simultaneous addition of nitrogen and water reduced pH by 7.22%, addition of water had no effect on pH.

Table 1. The F-values obtained from two-way ANOVAs for the impact of water (W), nitrogen (N), and the interaction between N × W on APA: acid phosphatase, BG: β-1,4-glucosidase, TC: total carbon, TN: total nitrogen, TP: total phosphorus, AP: available phosphorus, IN: inorganic nitrogen, NO_3^-−N: nitrate nitrogen, NH_4^+−N: ammonium nitrogen, and TC:TN: the ratios of total carbon to total nitrogen and TN;TP: total nitrogen to total phosphorus.

	N	**W**	**N × W**
APA	19.21 ***	11.87 **	5.98 *
BG	3.35	2.71	0.39
SM	2.09	74.82 ***	0.01
ST	30.66 ***	33.86 ***	2.33
pH	199.77 ***	35.79 ***	9.74 **
TC	1.13	3.79	0.27
TN	0.72	1.97	0.17
TP	19.46 ***	0.71	6.38 *
AP	0.19	3.25	0.73
IN	5.46 *	0.92	3.70
NO_3^-−N	11.30 **	2.47	0.41
NH_4^+−N	0.32	5.23 *	3.79.
TC:TN	0.41	3.90	0.25
TN:TP	15.51 ***	1.13	7.91 **
MBC	4.56 *	0.63	0.52
MBN	0.94	0.64	8.15 **
MBC:MBN	1.95	2.38	1.38

Note: * indicate $p < 0.05$, ** indicate $p < 0.01$, and *** indicate $p < 0.001$.

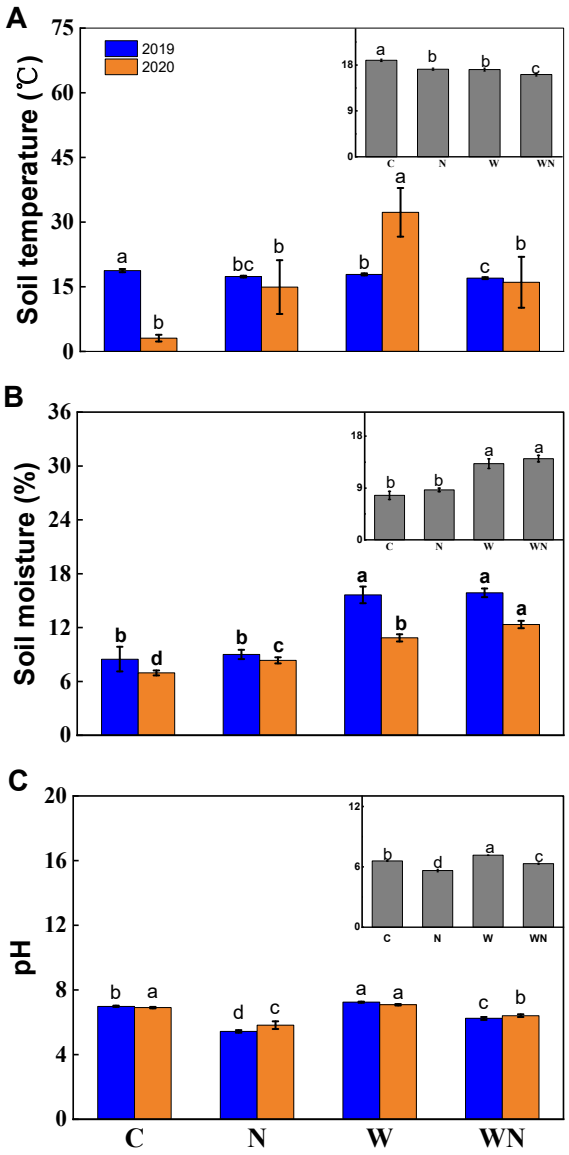


Figure 2. In 2019 and 2020, the effects of N and W on soil temperature (**A**), soil moisture (**B**), and pH (**C**) were observed. Distinct lowercase letters signify variation among treatments ($p < 0.05$). The insets indicate the average values for the two years of sampling. C, control; N, nitrogen addition; W, water addition; and WN, nitrogen addition plus water addition.

Nitrogen and water addition had no impact on TC or TN (Table 1, Figure 3A, B). There was a significant impact on TP after nitrogen addition ($p < 0.001$, Table 1). Nitrogen and water had an interactive effect on TP ($p < 0.05$) and reduced TP (Figure 3C). The addition of nitrogen and water did not have any impact on AP, as shown in Table 1 and Figure 3D. Nitrogen addition significantly affected IN ($p < 0.05$, Table 1), and adding nitrogen increases inorganic nitrogen (Figure 3E). Nitrogen addition significantly affected NO_3^-−N ($p < 0.05$, Table 1), and the NO_3^-−N showed a significant increase due to the nitrogen addition treatments (Figure 3F). Water addition significantly affected NH_4^+−N ($p < 0.01$, Table 1). The addition of water significantly increased the NH_4^+−N ($p < 0.05$, Figure 3G). In 2019, the addition of nitrogen and water had no effect on NH_4^+−N. In 2020, the addition of nitrogen had no effect on NH_4^+−N, Water addition increased NH_4^+−N ($p < 0.05$). Both nitrogen and water had no effect on TC:TN (Table 1, Figure 3H). Nitrogen addition significantly affected TN:TP ($p < 0.001$), and nitrogen and water had an interactive effect on TN:TP ($p < 0.01$). The addition of nitrogen increased TN:TP ($p < 0.05$, Figure 3I), while the addition

of water also increased TN:TP ($p < 0.05$), and simultaneous addition of nitrogen and water increased TN:TP ($p < 0.05$).

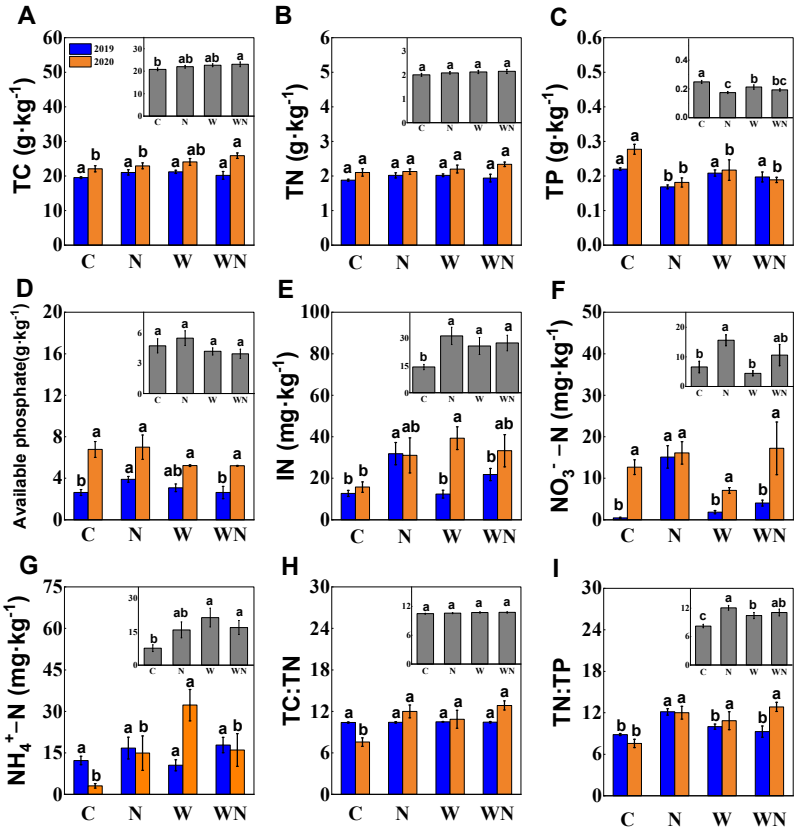


Figure 3. Effects of nitrogen and water on the physical and chemical characteristics of the soil. Distinct lowercase letters signify variation among treatments ($p < 0.05$). The insets indicate the average values for the years 2019 and 2020. The study included measurements of TC: total carbon (**A**), TN: total nitrogen (**B**), TP: total phosphorus (**C**), AP: available phosphorus (**D**), IN: inorganic nitrogen (**E**), NO_3^-−N: nitrate nitrogen (**F**), NH_4^+−N: ammonium nitrogen (**G**), TC:TN: the ratios of total carbon to total nitrogen (**H**), and TN:TP: the ratios of total nitrogen to total phosphorus (**I**).

3.2. Effect of Nitrogen Addition and Water Addition on Enzyme Activity and Microbial Biomass

Nitrogen addition had a tendency to inhibit MBC (Table 1, Figure 4A), with nitrogen addition decreasing MBC by 14.39% and water addition increasing MBC by 15.53%. Simultaneous addition of nitrogen and water had no effect on MBC. In 2019, nitrogen reduced MBC by 16.88%, while water addition increased MBC by 18.00%. In 2020, nitrogen reduced MBC by 9.61% and water addition increased MBC by 10.80%.

Nitrogen addition promoted MBN (Table 1, Figure 4B), nitrogen addition increased MBN by 51.44%, water addition had a tendency to promote MBN increases, water addition increased MBN by 36.06%, and simultaneous addition of nitrogen and water had no effect on MBN. In 2019, neither nitrogen nor water had an effect on MBN. But in 2020, nitrogen addition significantly promoted MBN by 100.92% ($p < 0.05$), while water addition showed a trend of promoting MBN by 84.77%. Both nitrogen and water additions had an effect on MBC:MBN (Table 1, Figure 4C). However, nitrogen addition significantly reduced MBC:MBN by 37.72% in 2019 ($p < 0.05$), and water addition significantly promoted MBC:MBN by5.37% ($p < 0.05$), the addition of nitrogen and water had no effect on MBC:MBN. In 2020, nitrogen addition significantly reduced MBC: MBN by 69.74% ($p < 0.05$), while water addition significantly reduced MBC:MBN by 43.67% ($p < 0.05$). The simultaneous addition of nitrogen and water to MBC:MBN had no effect ($p > 0.05$).

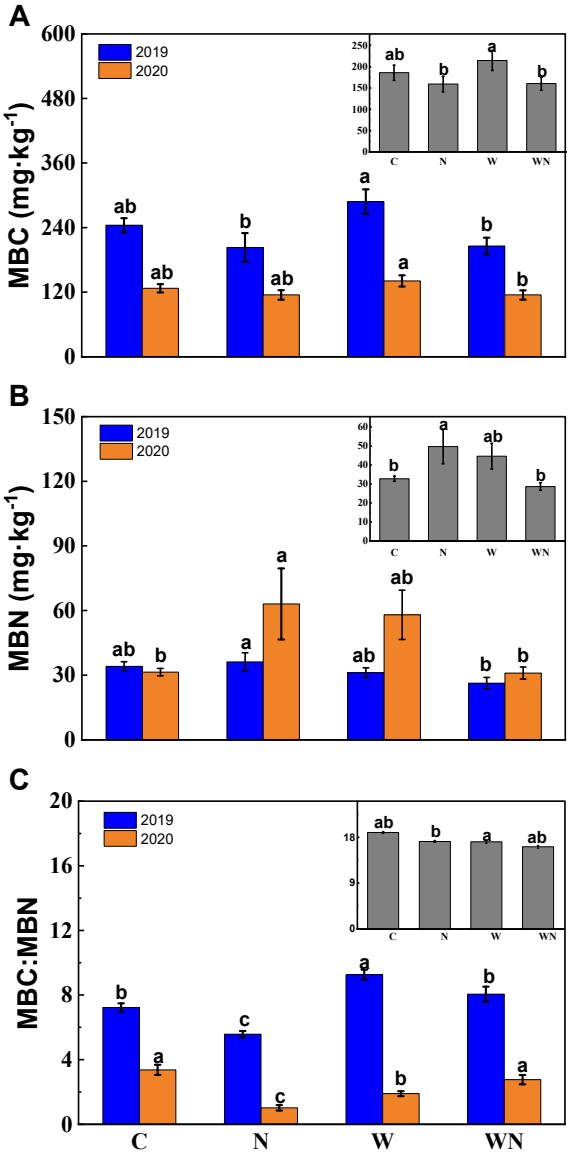


Figure 4. The impact of N and W on MBC: soil microbial biomass carbon (**A**), MBN: soil microbial biomass nitrogen (**B**), and MBC:MBN: the ratios of MBC to MBN (**C**). Treatments with distinct lowercase letters indicate a significant difference ($p < 0.05$). The insets indicate the average values for the years 2019 and 2020.

Although BG activity did not respond to nitrogen addition or water addition conditions (Tables 1 and 2, Figure 5A), nitrogen addition showed an inhibitory trend on BG compared to the control. The addition of nitrogen reduced BG by 7.90%, but the addition of water promoted BG by 14.93%, the addition of nitrogen and water simultaneously decreased BG by 1.21%. The trend of BG in 2019 and 2020 was similar. In 2019, the addition of nitrogen and water had no effect on BG, the addition of water increased BG by 15.95%, and the simultaneous addition of nitrogen and water decreased BG by 18.92%. In 2020, the effects of nitrogen and water were different, with nitrogen addition significantly inhibiting BG by 16.06% ($p < 0.05$). Water addition had a tendency to promote BG, with water addition increasing BG by 13.93%, while nitrogen and water addition simultaneously increased BG by 16.16%.

The effect of adding water on APA activity varied over time (Tables 1 and 2, Figure 5B), and nitrogen and water interacted to affect APA activity. Both nitrogen ($p < 0.001$) and water significantly inhibited APA ($p < 0.01$, Table 1). APA activity decreased by 9.86% with nitrogen addition ($p < 0.05$, Figure 4B), and decreased by 24.45% with water addition ($p < 0.05$, Figure 4B). In 2019, the addition of nitrogen inhibited APA by 24.07%, while the

addition of water had no effect on APA. The simultaneous addition of nitrogen and water inhibited APA, resulting in a decrease in APA by 37.52% ($p < 0.05$). In 2020, both nitrogen and water inhibited APA, and decreased by 75.27% and 80.76%, respectively ($p < 0.05$). The simultaneous addition of nitrogen and water reduced APA by 76.95%.

Table 2. Results (*F*-values) of linear mixed-effects models with a repeated measures split-plot design on the effects of year (Y), water (W) addition, nitrogen (N) addition, and their interactions on BG and APA activity.

	Y	N	W	N × W	N × Y	W × Y
BG	0.71	3.23	3.63	0.29	0.49	3.77
APA	99.06 ***	80.75 ***	57.07 ***	28.67 ***	1.43	21.66 ***

Note: *** indicate $p < 0.001$.

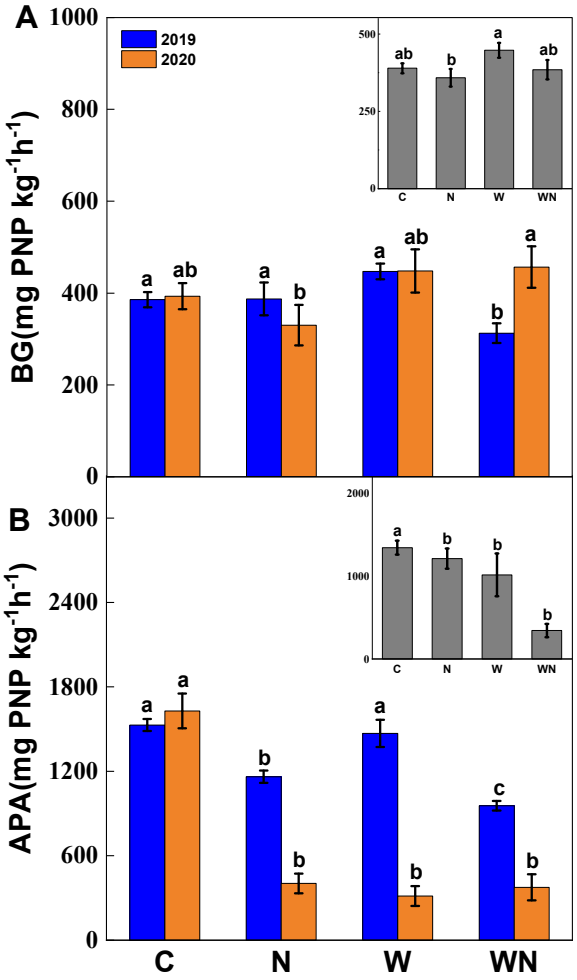


Figure 5. Effects of the addition of nitrogen and water on the activities of β-1,4-glucosidase (**A**) and acid phosphatase in soil (**B**). Significant differences are indicated by small lowercase letters ($p < 0.05$). The average value of each sample year is represented by each inset.

3.3. Key Factors Driving Changes in Soil Enzyme Activity

To better understand the links of soil physicochemical properties to BG and APA, linear regressions were plotted. The results showed that BG was significantly correlated with TC, TN, IN, NH_4^+−N, MBC, and MBN (Figure S2). APA was significantly correlated with SM, ST, pH, TP, IN NO_3^-−N, NH_4^+−N, TN:TP, and MBC (Figure S3).

In Figure 6, we employed RDA to differentiate the influence of environmental factors on soil enzyme activity. BG and APA were used as biological variables, while soil physical

and chemical property indicators were used as environmental variables. The results showed that the RDA1 and RDA2 of soil physicochemical properties explained 55.53% and 0.82% of soil enzyme activity varies, respectively. Therefore, the alterations in enzyme function were primarily influenced by the first axis. The results showed that MBC had the strongest effect on enzyme activity 24.2% (p = 0.002), TN:TP explained 16.7% (p = 0.002) and NH_4^+−N (p = 0.008) explained 7.6% of variations in soil enzyme activity (Table S1).

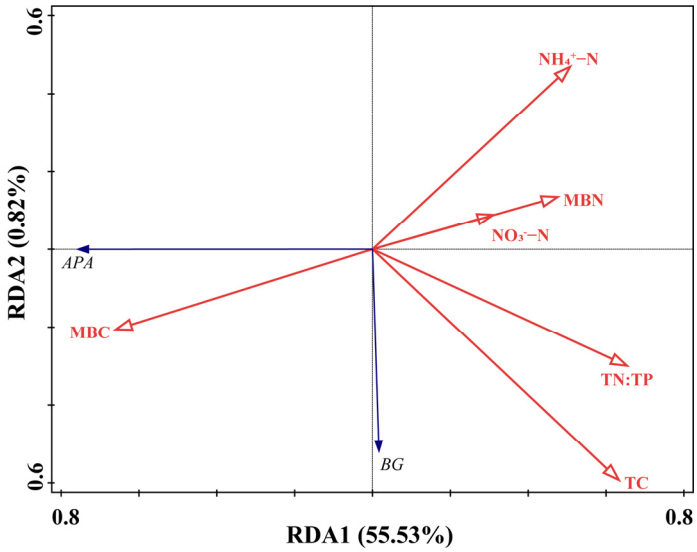


Figure 6. Analysis of soil enzyme activity and chemical factors using RDA.

4. Discussion

4.1. Effects of Nitrogen and Water Addition on Soil Enzyme Activity

The nutrient needs of microorganisms [29] and their functional response to environmental changes [9] can be evaluated by measuring soil enzyme activity. BG and APA, as components of soil extracellular enzymes, are often used to elucidate changes in C and P cycling processes in response to environmental changes [12,13]. Previous studies found that nitrogen addition significantly affected enzyme activity associated with soil P-conversion [13,14,16]. Our results showed that nitrogen addition inhibited APA (Table 1, Figure 5A,B), which was line with some empirical studies that nitrogen addition reduced soil enzyme activity [35,36]. The main reason for these phenomena was that the addition of nitrogen in the external environment leads to an increase in inorganic nitrogen in the soil, which reacts chemically with soil organic matter to produce substances that are unavailable to microorganisms [10], thus reducing microbial activity. Another study found that microbial inhibition was more common in fertilizer treatments over a five-year experiment [37]. The addition treatments of nitrogen and water for 16 years demonstrated that long-term nitrogen addition resulted in soil acidification (Figure 2C), which is harmful to the activity of enzymes in the soil, leading to a decrease in soil enzyme activity [38]. At the same time, long-term nitrogen application can damage soil aggregates, destroy the living environment of microorganisms, and inhibit microbial activity [39]. Our results showed that nitrogen addition had no effect on BG (Table 1, Figure 5A,B). The possible reason for this phenomenon is the long residence time required for nutrients in the soil and the untimely feedback to nitrogen [5,17].

The addition of water was determined to have no notable impact on BG activity, except for a significant decrease in APA activity in 2020. Most previous studies showed that water addition would not affect most soil enzyme activities [15]. As the feedback of soil enzyme activity to water is related to the pre-soil moisture content [40], and there is a threshold of microbial community response to moisture content [34]. Once the threshold is exceeded, increased soil moisture can lead to nutrient leaching and reduction in microbial resource use, and therefore soil microbial enzyme activity [5]. A study showed that

β-1,4-glucosidase activity did not increase with increasing precipitation, and changes in soil water content also affected microbial intracellular and extracellular pressures [41], which in turn affected the release of soil enzymes to the environment [42]. Our results showed a significant reduction in APA activity for the water augmentation treatment in 2020 (Figure 5B); this may be related to annual precipitation. Our study found that annual precipitation in 2020 was 410 mm, significantly higher than the historical average annual precipitation of 379 mm (Figure S1). High precipitation can easily create a hypoxic environment for the soil, thereby inhibiting APA activity [12]. In addition, adding water can improve the physical and chemical properties of the soil, as well as increase the total and available phosphorus content of the soil, resulting in soil microorganisms being in a state of phosphorus nutrient 'enrichment' [43], which in turn reduces the secretion or activity of phosphorus nutrient-related enzymes.

4.2. Key Factors Driving Soil Enzyme Activity

The RDA results indicated that the main indicator factor affecting soil enzyme activity was MBC (Figure 6), which was positively correlated with BG and APA, indicating that MBC stimulated enzyme activity. It is reported that MBC plays the most important role in the soil carbon–phosphorus cycle [44]. The number of soil microorganisms can to some extent indicate the vigorous level of material metabolism in the soil, which is a comprehensive reflection of soil physical and chemical properties and an important indicator of soil fertility. Most soil enzymes are derived from microorganisms and other organic tissues (animals, plants, organisms, and their residues), so the number of soil microorganisms is closely related to soil enzyme activity. In addition, Allison et al. [45] discussed the importance of microbial physiology and biomass in the soil carbon and nitrogen cycle under climate change and found that microbial biomass and enzyme activity are affected by climate change in the same direction. With the increase in enzyme activity and microbial biomass, the soil carbon cycle was affected differently. However, when enzyme activity and microbial biomass adapt to climate change, the effect of climate change on the carbon cycle is reduced. In this study, nitrogen addition reduced MBC and enzyme activity, which was consistent with previous studies. Yang et al. [46] found that the presence of soil microbial biomass carbon has a significant impact on soil enzyme activity, with enzyme activity showing an increase as microbial biomass carbon levels rise. In summary, the direction of change in microbial biomass carbon and enzyme activity was consistent.

There was a negative correlation between NH_4^+-N and both BG and APA, indicating that NH_4^+-N inhibits the activity of the enzymes. The effect of NH_4^+-N on carbon and phosphorus cycling enzyme activity is enhanced by nitrogen application, and NH_4^+-N has a stronger effect on soil acidification than NO_3^--N. Water addition was shown to increase NH_4^+-N in the soil and react with soil organic matter to produce indoles and pyrroles [47], which are difficult for microorganisms to utilize [48], thereby inhibiting microbial activity. In addition, NH_4^+-N often displaces salt-based ions (Ca^{2+}, Mg^{2+}, K^+, and Na^+) from the surface of soil colloids, making them susceptible to leaching. When NH_4^+-N is taken up by plants, it releases H^+ into the soil solution, thus causing soil acidification [49], which is unsuitable for microbial survival. TN:TP is negatively correlated with APA, indicating that APA decreases as TN:TP increases, suggesting that soil stoichiometry may be an important driver of soil enzyme activity. Ecological stoichiometric ratios can indicate constraints to biogeochemical cycling as a predictor of carbon cycling [50,51]. Sinsabaugh et al. [48] found that soil enzyme activity is sensitive to soil stoichiometry (C:N:P) [52]. It was suggested that the effect of soil stoichiometry on enzyme activity is mainly through the maintenance of stoichiometric balance by microorganisms [43]. Soil stoichiometry can strongly influence the structure and activity of microbial communities and regulate the metabolism of microbial communities, leading to changes in soil enzyme activity [53–55].

5. Conclusions

A field experiment based on 16 years of nitrogen and precipitation manipulated in temperate grassland in northern China, our study showed that nitrogen addition decreased both BG and APA, and that water addition only inhibited APA in 2020. Nitrogen and water addition affected enzyme activity mainly by affecting MBC, NH_4^+−N, and TN:TP, with MBC having the most important effect. In general, the addition of nitrogen or water over a long-term of time did not affect BG, suggesting that BG is a resilient enzyme that remains unaffected by changes in the environment. In contrast, APA is sensitive to nitrogen and water addition and responds dramatically to interannual fluctuations in precipitation, suggesting that APA can be used as an indicator of changes in soil nutrient cycling due to climate change. However, our study used only BG and APA to explore the effects of environmental changes and their implications on carbon and phosphorus cycling. Since different enzymes could respond differently to environmental variations, the representativeness of only one enzyme on carbon or phosphorus cycling is not convincing enough, which warrants further investigations on more enzymes in future studies. Although based on the long-term experiment of nitrogen and water addition, our sampling on enzyme activity was only conducted for two years, and the interannual variation was rather great. Thus, long-term monitoring is needed in future studies to reveal the more reliable patterns and mechanisms of enzyme activity in response to global change in the semi-arid grassland.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13071920/s1. Figure S1. Natural precipitation in 2019 and 2020 compared to historical average precipitation (2005–2020). Figure S2. Linear regressions between β-1,4-glucosidase (BG) activities and soil physicochemical properties. Total carbon (TC), total nitrogen (TN), inorganic nitrogen (IN), ammonium nitrogen (NH_4^+−N), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN). The lines represent significant relationships ($p < 0.05$). Red represents 2019, blue represents 2020. Figure S3. Linear regressions between acid phosphatase (APA) activities and soil physicochemical properties. The lines represent significant relationships ($p < 0.05$). Total phosphorus (TP), inorganic nitrogen (IN), nitrate nitrogen (NO_3^-−N), ammonium nitrogen (NH_4^+−N), total nitrogen to total phosphorus (TN:TP), microbial biomass carbon (MBC). Red represents 2019, blue represents 2020. Table S1. Percentage of variation in soil enzymatic activity explained by soil variables.

Author Contributions: Conceptualization, Z.X., K.J. and J.Z. (Jinbao Zhang); methodology, J.Z. (Jinbao Zhang), Y.L. and R.T.; field and laboratory work, J.Z. (Jinbao Zhang), S.W., R.T., Y.S., J.Z. (Jiatao Zhang), N.L. and W.S.; analysis, Y.S., L.D., W.S. and J.Z. (Jiatao Zhang); writing—original draft preparation, J.Z. (Jinbao Zhang), L.D. and Z.X.; writing—review and editing, J.Z. (Jinbao Zhang) and Z.X.; funding acquisition, Z.X. All authors have read and agreed to the published version of the manuscript.

Funding: This work was jointly supported by the National Key Research and Development Program of China (2022YFF1302300), and the National Natural Science Foundation of China (32060284).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Galloway, J.N.; Townsend, A.R.; Erisman, J.W.; Bekunda, M.; Cai, Z.; Freney, J.R.; Martinelli, L.A.; Seitzinger, S.P.; Sutton, M.A. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* **2008**, *320*, 889–892. [CrossRef] [PubMed]
2. IPCC. Summary for Policymakers. In *Climate Change 2021: The Physical Science Basis*; Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change; IPCC: Geneva, Switzerland, 2021.

3. Niu, G.X.; Wang, R.Z.; Hasi, M.Q.; Wang, Y.L.; Geng, Q.Q.; Wang, C.H.; Jiang, Y.; Huang, J.H. Availability of soil base cations and micronutrients along soil profile after 13-year nitrogen and water addition in a semi-arid grassland. *Biogeochemistry* **2021**, *152*, 223–236. [CrossRef]
4. Niu, S.L.; Yang, H.J.; Zhang, Z.; Wu, M.Y.; Lu, Q.; Li, L.H.; Han, X.G.; Wan, S.Q. Non-Additive Effects of Water and Nitrogen Addition on Ecosystem Carbon Exchange in a Temperate Steppe. *Ecosystems* **2009**, *12*, 915–926. [CrossRef]
5. Wang, R.Z.; Creamer, C.A.; Wang, X.; He, P.; Xu, Z.W.; Jiang, Y. The effects of a 9-year nitrogen and water addition on soil aggregate phosphorus and sulfur availability in a semi-arid grassland. *Ecol. Indic.* **2016**, *61*, 806–814. [CrossRef]
6. Yang, K.; Zhu, J.J.; Gu, J.; Yu, L.Z.; Wang, Z.Z. Changes in soil phosphorus fractions after 9 years of continuous nitrogen addition in a Larix gmelinii plantation. *Ann. For. Sci.* **2014**, *72*, 435–442. [CrossRef]
7. Deng, L.; Peng, C.H.; Huang, C.B.; Wang, K.B.; Liu, Q.Y.; Liu, Y.L.; Hai, X.Y.; Shangguan, Z.P. Drivers of soil microbial metabolic limitation changes along a vegetation restoration gradient on the Loess Plateau, China. *Geoderma* **2019**, *353*, 188–200. [CrossRef]
8. Guan, P.T.; Yang, J.J.; Yang, Y.R.; Wang, W.; Zhang, P.; Wu, D.H. Land conversion from cropland to grassland alleviates climate warming effects on nutrient limitation: Evidence from soil enzymatic activity and stoichiometry. *Glob. Ecol. Conserv.* **2020**, *24*, e01328. [CrossRef]
9. Sinsabaugh, R.L.; Lauber, C.L.; Weintraub, M.N.; Ahmed, B.; Allison, S.D.; Crenshaw, C.; Contosta, A.R.; Cusack, D.; Frey, S.; Gallo, M.E.; et al. Stoichiometry of soil enzyme activity at global scale. *Ecol. Lett.* **2008**, *11*, 1252–1264. [CrossRef]
10. Zi, H.B.; Hu, L.; Wang, C.T.; Wang, G.X.; Wu, P.F.; Lerdau, M.; Ade, L.J. Responses of soil bacterial community and enzyme activity to experimental warming of an alpine meadow. *Eur. J. Soil Sci.* **2018**, *69*, 429–438. [CrossRef]
11. Kang, L.; Han, X.; Sun, Z.O.J. Biological Science in China | | Grassland Ecosystems in China: Review of Current Knowledge and Research Advancement. *Philos. Trans. R. Soc. B* **2007**, *362*, 997–1008. [CrossRef]
12. Hewins, D.B.; Broadbent, T.; Carlyle, C.N.; Bork, E.W. Extracellular enzyme activity response to defoliation and water addition in two ecosites of the mixed grass prairie. *Agric. Ecosyst. Environ.* **2016**, *230*, 79–86. [CrossRef]
13. Chen, J.; Luo, Y.; Li, J.; Zhou, X.; Zhou, L. Costimulation of soil glycosidase activity and soil respiration by nitrogen addition. *Glob. Chang. Biol.* **2016**, *23*, 1328–1337. [CrossRef] [PubMed]
14. Jing, X.; Yang, X.; Ren, F.; Zhou, H.; Zhu, B.; He, J.S. Neutral effect of nitrogen addition and negative effect of phosphorus addition on topsoil extracellular enzymatic activities in an alpine grassland ecosystem. *Appl. Soil Ecol.* **2016**, *107*, 205–213. [CrossRef]
15. Ren, C.J.; Zhao, F.Z.; Shi, Z.; Chen, J.; Han, X.H.; Yang, G.H.; Feng, Y.Z.; Ren, G.X. Differential responses of soil microbial biomass and carbon-degrading enzyme activities to altered precipitation. *Soil Biol. Biochem.* **2017**, *115*, 1–10. [CrossRef]
16. Xiao, W.; Chen, X.; Jing, X.; Zhu, B.A. A meta-analysis of soil extracellular enzyme activities in response to global change. *Soil Biol. Biochem.* **2018**, *123*, 21–32. [CrossRef]
17. Ma, W.; Li, J.; Gao, Y.; Xing, F.; Sun, S.; Zhang, T.; Zhu, X.; Chen, C.; Li, Z. Responses of soil extracellular enzyme activities and microbial community properties to interaction between nitrogen addition and increased precipitation in a semi-arid grassland ecosystem. *Sci. Total Environ.* **2020**, *703*, 134691. [CrossRef]
18. Keeler, B.L.; Hobbie, S.E.; Kellogg, L.E. Effects of Long-Term Nitrogen Addition on Microbial Enzyme Activity in Eight Forested and Grassland Sites: Implications for Litter and Soil Organic Matter Decomposition. *Ecosystems* **2009**, *12*, 1–15. [CrossRef]
19. Gong, S.; Zhang, T.; Guo, R.; Cao, H.; Shi, L.; Guo, J.; Sun, W. Response of soil enzyme activity to warming and nitrogen addition in a meadow steppe. *Soil Res.* **2015**, *53*, 242. [CrossRef]
20. Yue, K.; Fornara, D.A.; Yang, W.; Peng, Y.; Li, Z.; Wu, F.; Peng, C. Effects of three global change drivers on terrestrial C:N:P stoichiometry: A global synthesis. *Glob. Chang. Biol.* **2016**, *23*, 2450–2463. [CrossRef]
21. Peng, X.Q.; Wang, W. Stoichiometry of soil extracellular enzyme activity along a climatic transect in temperate grasslands of northern China. *Soil Biol. Biochem.* **2016**, *98*, 74–84. [CrossRef]
22. Xu, Z.W.; Wan, S.Q.; Zhu, G.L.; Ren, H.Y.; Han, X.G. The Influence of Historical Land Use and Water Availability on Grassland Restoration. *Restor. Ecol.* **2010**, *18*, 217–225. [CrossRef]
23. Zeglin, L.H.; Stursova, M.; Sinsabaugh, R.L.; Collins, S.L. Microbial responses to nitrogen addition in three contrasting grassland ecosystems. *Oecologia* **2007**, *154*, 349–359. [CrossRef]
24. Lei, L.; Gundersen, P.; Tao, Z.; Mo, J. Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. *Soil Biol. Biochem.* **2012**, *44*, 31–38.
25. Han, Z.G.; Xin, S.; Li, M.H. Effects of Forest Age on Soil Fungal Community in a Northern Temperate Ecosystem. *Indian J. Microbiol.* **2016**, *56*, 328–334.
26. Li, G.; Kim, S.; Han, S.H.; Chang, H.; Du, D.L.; Son, Y. Precipitation affects soil microbial and extracellular enzymatic responses to warming. *Soil Biol. Biochem.* **2018**, *120*, 212–221. [CrossRef]
27. Olander, L.P.; Vitousek, P.M. Regulation of soil phosphatase and chitinase activityby N and P availability. *Biogeochemistry* **2000**, *49*, 175–190. [CrossRef]
28. Vitousek, P.M.; Porder, S.; Houlton, B.Z.; Chadwick, O.A. Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen-phosphorus interactions. *Ecol. Appl.* **2010**, *20*, 5–15. [CrossRef]
29. Moorhead, D.L.; Sinsabaugh, R.L. A theoretical model of litter decay and microbial interaction. *Ecol. Monogr.* **2006**, *76*, 151–174. [CrossRef]
30. Chung, H.G.; Zak, D.R.; Reich, P.B.; Ellsworth, D.S. Plant species richness, elevated CO2, and atmospheric nitrogen deposition alter soil microbial community composition and function. *Glob. Chang. Biol.* **2007**, *13*, 980–989. [CrossRef]

31. Sun, Y.; Ding, Y. A projection of future changes in summer precipitation and monsoon in East Asia. *Sci. China Earth Sci.* **2010**, *53*, 284–300. [CrossRef]
32. Bai, Y.F.; Wu, J.G.; Clark, C.M.; Naeem, S.; Pan, Q.M.; Huang, J.H.; Zhang, L.X.; Han, X.G. Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: Evidence from inner Mongolia Grasslands. *Glob. Chang. Biol.* **2010**, *16*, 358–372. [CrossRef]
33. Zhang, Y.; Zheng, L.; Liu, X.; Jickells, T.; Cape, J.N.; Goulding, K.; Fangmeier, A.; Zhang, F. Evidence for organic N deposition and its anthropogenic sources in China. *Atmos. Environ.* **2008**, *42*, 1035–1041. [CrossRef]
34. Henry, H.A.L. Soil extracellular enzyme dynamics in a changing climate. *Soil Biol. Biochem.* **2012**, *47*, 53–59. [CrossRef]
35. Kemmitt, S.J.; Wright, D.; Goulding, K.W.T.; Jones, D.L. pH regulation of carbon and nitrogen dynamics in two agricultural soils. *Soil Biol. Biochem.* **2006**, *38*, 898–911. [CrossRef]
36. Treseder, K.K. Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. *Ecol. Lett.* **2010**, *11*, 1111–1120. [CrossRef]
37. Li, L. Effects of land-use change on soil carbon storagein grassland ecosystems. *Sheng Tai Xue Bao* **1998**, *22*, 300–302.
38. Kang, H.; Freeman, C. Phosphatase and arylsulphatase activities in wetland soils: Annual variation and controlling factors. *Soil Biol. Biochem.* **1999**, *31*, 449–454. [CrossRef]
39. Bell, T.H.; Klironomos, J.N.; Henry, H.A.L. Seasonal Responses of Extracellular Enzyme Activity and Microbial Biomass to Warming and Nitrogen Addition. *Soil Sci. Soc. Am. J.* **2010**, *74*, 820–828. [CrossRef]
40. Thorn, K.A.; Mikita, M.A. Ammonia fixation by humic substances: A nitrogen-15 and carbon-13 NMR study. *Sci. Total Environ.* **1992**, *113*, 67–87. [CrossRef]
41. Zhou, X.Q.; Chen, C.R.; Wang, Y.F.; Xu, Z.H.; Han, H.Y.; Li, L.H.; Wan, S.Q. Warming and increased precipitation have differential effects on soil extracellular enzyme activities in a temperate grassland. *Sci. Total Environ.* **2013**, *444*, 552–558. [CrossRef]
42. Joshua, S.; Basler, T.C.; Matthew, W. Microbial stress-response physiology and its implicationsfor ecosystem function. *Ecology* **2007**, *88*, 1386–1394.
43. Burns, R.G.; DeForest, J.L.; Marxsen, J.; Sinsabaugh, R.L.; Stromberger, M.E.; Wallenstein, M.D.; Weintraub, M.N.; Zoppini, A. Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biol. Biochem.* **2013**, *58*, 216–234. [CrossRef]
44. Schimel, J.P.; Weintraub, M.N. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: A theoretical model. *Soil Biol. Biochem.* **2003**, *35*, 549–563. [CrossRef]
45. Allison, S.D.; Wallenstein, M.D.; Bradford, M.A. Soil-carbon response to warming dependent on microbial physiology. *Nat. Geosci.* **2010**, *3*, 336–340. [CrossRef]
46. Yang, S.; Xu, Z.; Wang, R.; Zhang, Y.; Li, H. Variations in soil microbial community composition and enzymatic activities in response to increased N deposition and precipitation in Inner Mongolian grassland. *Appl. Soil Ecol.* **2017**, *119*, 275–285. [CrossRef]
47. Dail, D.B.; Davidson, E.A.; Chorover, J. Rapid abiotic transformation of nitrate in an acid forest soil. *Biogeochemistry* **2001**, *54*, 131–146. [CrossRef]
48. Sinsabaugh, R.L.; Hill, B.H.; Shah, J.J.F. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature* **2009**, *462*, 795–798. [CrossRef]
49. Kivlin, S.N.; Treseder, K.K. Soil extracellular enzyme activities correspond with abiotic factors more than fungal community composition. *Biogeochemistry* **2014**, *117*, 23–37. [CrossRef]
50. Ollinger, S.; Smith, M.; Martin, M.; Hallett, R.; Goodale, C.L. Regional variation in foliar chemistry and n cycling among forests of diverse history. *Ecology* **2002**, *83*, 339–355.
51. Xu, Z.; Yu, G.; Zhang, X.; He, N.; Wang, Q.; Wang, S.; Wang, R.; Zhao, N.; Jia, Y.; Wang, C. Soil enzyme activity and stoichiometry in forest ecosystems along the North-South Transect in eastern China (NSTEC). *Soil Biol. Biochem.* **2017**, *104*, 152–163. [CrossRef]
52. Xie, X.; Pu, L.; Wang, Q.; Zhu, M.; Xu, Y.; Zhang, M. Response of soil physicochemical properties and enzyme activities to long-term reclamation of coastal saline soil, Eastern China. *Sci. Total. Environ.* **2017**, *607–608*, 1419–1427. [CrossRef] [PubMed]
53. Cuia, Y.; Fanga, L.; Guoc, X.; Xia, W.; Zhangd, Y. Ecoenzymatic stoichiometry and microbial nutrient limitation in rhizosphere soil in the arid area of the northern Loess Plateau, China. *Soil Biol. Biochem.* **2018**, *116*, 11–21. [CrossRef]
54. Baquerizo, M.D.; Reich, P.B.; Khachane, A.N.; Campbell, C.D.; Thomas, N.; Freitag, T.E.; Alsoud, W.A.; Sorensen, S.J.; Bardgett, R.D.; Singh, B.K. It is elemental: Soil nutrient stoichiometry drives bacterial diversity. *Environ. Microbiol.* **2016**, *19*, 1176. [CrossRef] [PubMed]
55. Bowles, T.M.; Acosta-Martinez, V.; Calderon, F.; Jackson, L.E. Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. *Soil Biol. Biochem.* **2014**, *68*, 252–262. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.