agronomy

MDPI

Communication

The Effect of Molybdenum Fertilizer on the Growth of Grass–Legume Mixtures Related to Symbiotic Rhizobium

Jing Zhou [1], Xiao Sun [2], Chao Chen [1] and Jihui Chen [1,*]

[1] College of Animal Science, Guizhou University, Guiyang 550025, China
[2] College of Agro-Grassland Science, Nanjing Agricultural University, Nanjing 210095, China
* Correspondence: jhchen3@gzu.edu.cn

Abstract: Molybdenum (Mo) is required by the enzymes involved in many metabolic processes related to plant growth and development. However, the effects of Mo addition on plant growth and beneficial microorganisms in mixed grasslands are unclear. We conducted a greenhouse experiment to examine the effects of different Mo addition levels (10 and 20 mg Mo kg^{-1} soil in the form of Na_2MoO_4) on the growth of perennial ryegrass–white clover in two low-Mo soils, as well as their symbiotic microorganisms. Our results showed that the addition of Mo had a significant impact on plant growth in limestone soil but not in yellow loam soil ($p < 0.05$). Compared with no addition of Mo fertilizer in limestone soil, an addition of 10 mg Mo kg^{-1} significantly increased the plant community shoot and root biomass ($p < 0.05$). However, this improvement was not observed with an addition of 20 mg Mo kg^{-1}. The shoot nitrogen and phosphorus content in both soil types was unaffected by the Mo addition ($p > 0.05$), whereas the 10 mg Mo kg^{-1} addition significantly increased the shoot nitrogen and phosphorus uptake in limestone soil ($p < 0.05$). This increase in plant community productivity was primarily due to the increased growth of both species, caused by the enhanced activation of the symbiotic rhizobium. We conclude that Mo supply may promote N utilization and uptake in mixed grassland by increasing the activity of symbiotic rhizobium, resulting in a higher yield of mixed grassland, which is critical for sustainable agricultural development in low-Mo soils.

Keywords: molybdenum fertilizer; symbiotic rhizobium; forage quality; grassland productivity

check for updates

Citation: Zhou, J.; Sun, X.; Chen, C.; Chen, J. The Effect of Molybdenum Fertilizer on the Growth of Grass–Legume Mixtures Related to Symbiotic Rhizobium. *Agronomy* **2023**, *13*, 495. https://doi.org/10.3390/agronomy13020495

Academic Editor: Alwyn Williams

Received: 27 December 2022
Revised: 3 February 2023
Accepted: 7 February 2023
Published: 8 February 2023

1. Introduction

Molybdenum (Mo) is an essential micronutrient for the growth and development of higher plants, and it plays a vital role in the photosynthetic process [1–3]. It also plays a fundamental role in nitrogen (N) acquisition and assimilation by regulating biological N fixation and nitrate reductase activity [4,5]. Mo deficiency is common throughout the world, and approximately 47% of China's agricultural area is Mo deficient [6]. This issue is exacerbated in the acidic soil in southern China [7]. Available Mo in the soil is considered low if it is less than 0.11 mg kg^{-1} [8]: this can cause the disorganization of physiological and biochemical reactions in plant species, resulting in decreased plant growth and food quality [9–11]. However, it is unclear whether Mo addition affects forage yield and quality in pastures by enhancing the interactions between plants and their symbiotic root microorganisms.

Pasture systems based on forage mixtures of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) are commonly used in temperate regions due to high pasture productivity and quality, as well as their persistence under grazing management [12,13]. Numerous studies have found that Mo addition improves plant growth, particularly in legumes, by promoting a variety of physiological and biochemical processes [2,5,14]. However, some researchers have documented neutral or adverse effects of Mo addition on plant growth [15,16]. These inconsistencies could be attributed to the different Mo requirements of plants. Mo concentrations in gramineous plants range from 0.2 to 1 mg kg^{-1}, whereas leguminous plants have much higher concentrations, ranging from 0.5 to 20 mg kg^{-1} [17]. Leguminous plants also require more Mo than grass for N fixation [5]. Therefore, Mo fertilizers for low-Mo soil may improve the growth of legume plants in grass–legume mixed grasslands, which would also benefit the grass. Despite the critical role of grass–legume combinations in the ecosystem, most previous studies focused on single forages [14,18], and no general conclusions about the effect of Mo addition on plant growth have yet been drawn.

The high productivity and quality of grass–legume grasslands primarily benefit from symbiotic microorganisms [19–21]. Rhizobium requires ten times the amount of Mo that host legume protein synthesis does for nitrogen fixation [22]. Some studies have discovered that Mo fertilization in Mo-deficient soil has beneficial effects on rhizobium, including its nodule counts and weight [14,18,23]. Arbuscular mycorrhizal fungi (AMF) are essential, beneficial microorganisms that form mutualistic symbioses with most crops [24]. However, few previous studies on the effect of Mo addition on AMF have been conducted, with the exception of Shi et al. [25], who found that Mo additions had no significant effect on rates of root colonization. Therefore, different plant responses to Mo fertilization may be caused by differences in root symbiotic microorganisms, which have different Mo requirements. The absence of beneficial symbiotic microorganisms in some soils (for example, acidic soil) may also contribute to these inconsistencies. For these reasons, Mo deficiency in soil should reduce the function of symbiotic microorganisms, thus influencing the productivity of the grass–legume mixture grasslands. However, experimental studies exploring how much Mo addition affects the grass–legume mixture productivity by altering the functions of symbiotic microorganisms are still lacking.

In this study, a greenhouse experiment was conducted to investigate the effects of Mo fertilizer on the biomass and forage quality of artificial grassland in two types of low-Mo soil, and whether this correlates with nodulation, AMF, or both. We hypothesized that: (i) Mo addition can significantly improve the growth and quality of leguminous and gramineous plants in both soils; and (ii) the positive effects of Mo addition on plant growth and quality were primarily caused by an increase in symbiotic nodulation.

2. Materials and Methods

2.1. Experimental Design

In this study, we used a completely random design. A large amount of soil nutrients has been lost in the karst regions of Southwest China due to soil acidification and erosion, resulting in severe nutrient deficiencies, including Mo deficiency. Yellow loam soils and limestone are the main soil types in the karst area [26], Therefore, we collected two Mo-deficient soils from Guizhou, located in the southwestern karst area's core area (Table 1). Both the limestone soil and yellow loam soil were collected from natural grasslands in Dafang County and Huaxi County, respectively, and the biomass of the white clover and perennial mixed grassland in the two soils are 104.3 g m^{-2} and 338.4 g m^{-2}, respectively. Experimental treatments included the control and two levels of Mo treatments (10 and 20 mg Mo kg^{-1} soil in the form of Na_2MoO_4) (Macklin Biochemical Co., Ltd., Shanghai, China) for both types of soil, with four replications. We used 24 plastic pots (16 cm in diameter, 12 cm in depth) (HuanQiu, Yangzhou, China), each holding 1500 g of soil (average particle size of <2 mm). This study was conducted in a greenhouse at Guizhou University.

Table 1. Physico-chemical properties of the soil.

Characteristic	pH	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Available P (mg kg^{-1})	Available Mo (mg kg^{-1})	Total Mo (mg kg^{-1})
Limestone soil	5.79	30.28	4.51	2.89	0.10	1.77
Yellow loam soil	5.46	19.67	1.41	7.99	0.08	1.99

2.2. Plant Materials

White clover and perennial ryegrass are two of the most widely used forages for high-quality food for livestock. They are also used successfully in floor vegetation. Hence, we selected perennial ryegrass and white clover as experimental materials. In August 2021, each pot was sown with eight perennial ryegrass and eight white clover seeds at a depth of 2–3 mm in greenhouses with a relative humidity of 70%. The greenhouse was retained under a 20 °C/15 °C day (16 h)/night (8 h) temperature cycle. For each species, four seedlings were grown in each pot, and Mo fertilizer (10 and 20 mg Mo kg^{-1} soil in the form of Na_2MoO_4) was added. The fertilizer was dissolved in deionized water, which was then used to ensure uniform fertilizer application and aid plant absorption. In addition, considering the severe lack of soil P in the karst area, the same amount of P fertilizer (50 mg P kg^{-1} soil in the form of KH_2PO_3) (Tianjing Kermel Biochemical Co., Ltd., Tianjing, China) was added to each pot. The control group received the same treatment, but without Mo. Furthermore, we rearranged the pots on a weekly basis to ensure that the environmental surroundings were random and that any effect on plant growth was minimized.

2.3. Sampling and Chemical Analysis

The plants in each pot were harvested and separated from the soil after four months of growth, and completely washed with deionized water to remove any aerial deposition. Perennial ryegrass and white clover were harvested separately and divided into shoots and roots. Fresh plants were dried for 48 h at 65 °C to measure shoot dry weight. The roots were washed with tap water and dried under the same conditions as the shoots to measure root biomass. The effective root nodules were removed from the white clover roots, counted, and weighed. Given that both forages have the potential to be infested by AMF, we evaluated their infestation rates. The AMF colonization was then measured using approximately 0.5 g (fresh weight) of both plant roots (see below).

AMF Colonization measurement: The roots of white clover and perennial ryegrass used to determine the infection rate of AMF were each cut into 1 cm lengths and heated in a 90 °C water bath with a 10% potassium hydroxide solution (Tianjing Kermel Biochemical Co., Ltd., Tianjing, China) until transparent. Subsequently, they were treated with 1% hydrochloric acid solution (Chongqing Chuandong Chemical Co., Ltd., Chongqing, China), and finally dyed with 0.05% (w/v) Trypan blue (Beijing Solaibao Technology Co., Ltd., Beijing, China) for 30 min [27]. We then used the grid line intersection method to measure the root colonization rate. We randomly selected 30 root segments for each sample to evaluate the degree of AMF colonization under a dissecting microscope at ×40 magnification (Leica DM3000 LED, Wetzlar, Germany). For the glass slide method, 1 cm root segments were randomly selected to form stained root samples, mounted on microscope slides, and examined under a compound microscope(Leica DM3000 LED, Wetzlar, Germany).

Elemental Analyses of Shoot Samples: The shoot samples were ground to determine the nutritional levels, total P concentrations (mg g^{-1}), using the sodium hydrogen carbonate solution–Mo–Sb anti spectrophotometric method [28], and total N concentrations (mg g^{-1}) using an elemental analyzer (Vario EL III, Elementar, Langenselbold, Germany). N and P uptake were obtained by multiplying N and P concentration with the shoot biomass.

2.4. Statistical Analysis

A one-way ANOVA was used to determine the effects of Mo treatment on plant growth, nutrient concentrations and uptake, and root symbiotic microorganisms (rhizobia and AMF). The Duncan test was used to distinguish between treatments ($p \leq 0.05$). The Pearson correlation coefficient was used to analyze the relationship between the shoot biomass of the mixture and plant symbiotic microorganisms. We used SPSS 20.0 for Windows to analyze the data (IBM SPSS Inc. Chicago, IL, USA).

3. Results

3.1. Effects of Mo on Forage Growth

Mo addition had a significant effect on the shoot and root biomass of the perennial ryegrass and white clover mixtures in limestone soil ($p < 0.05$) but had no significant effect on the shoot and root biomass in yellow loam soil ($p > 0.05$) (Table 2, Figure 1A,B). In particular, when compared to the control in limestone soil, the addition of 10 mg Mo kg^{-1} increased the shoot and root biomass of the white clover and perennial ryegrass mixture by 25.5% and 40.3%, respectively. However, a 20 mg Mo kg^{-1} addition showed no significant influence on the biomass ($p > 0.05$). Furthermore, the effects of Mo addition on the shoot and root biomass for each forage were similar; both increased with a 10 mg Mo kg^{-1} addition; however, no significant effects were observed with further Mo addition (Figure S1A,B).

Table 2. F values from a one-way ANOVA for the Molybdenum addition for the total shoot biomass of mixture (SB), total root biomass of mixture (RB), total nitrogen (TN), total phosphorus (TP), nitrogen uptake (NU), phosphorus uptake (PU), nodule number (NN), nodule weight (NW), AMF colonization of ryegrass (AMF_1), and AMF colonization of white clover (AMF_2).

Soil Type	SB	RB	TN	TP	NU	PU	NN	NW	AMF_1	AMF_2
Limestone soil	28.10 ***	4.60 *	0.10	2.00	13.65 **	6.49 *	23.67 ***	10.22 **	0.00	3.00
Yellow loam soil	1.10	0.40	0.10	2.00	1.00	0.20	0.20	0.60	0.50	5.24 *

Asterisks denote significance: * = $p < 0.05$; ** = $p < 0.01$, *** = $p < 0.001$.

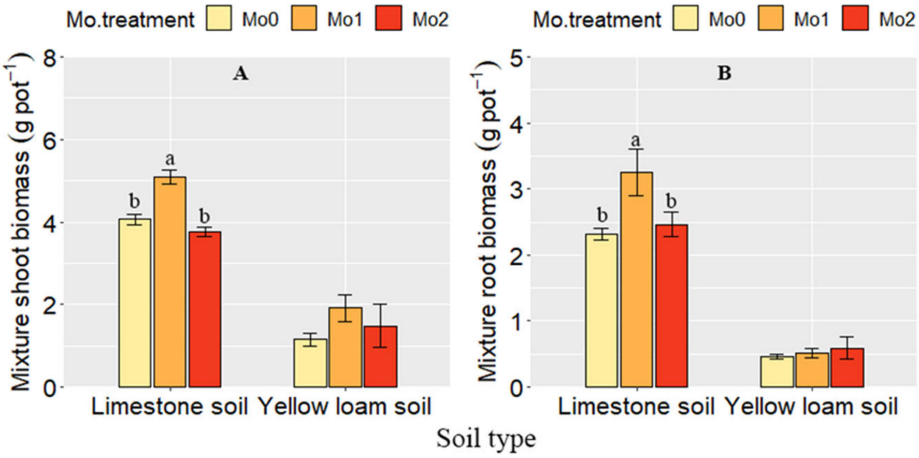

Figure 1. Influence of Molybdenum additions (Mo0, Mo1, and Mo2 represent 0, 10, and 20 mg Mo kg^{-1} soil in the form of Na_2MoO_4, respectively) to limestone soil and yellow loam soil on the shoot biomass (**A**) and root biomass (**B**) of the mixture (white clover and perennial ryegrass). Different lowercase letters represent multiple comparisons of means among treatments determined by Duncan-test. ($p < 0.05$).

3.2. Effects of Mo on the Quality of Forage

Across all Mo treatments, the mean values of the N and P concentrations in the white clover and perennial ryegrass mixture ranged from 18.67 to 23.95 mg g^{-1} and 1.59 to 2.36 mg g^{-1}, respectively. Mo addition had no significant effect on the N and P concentrations of the mixture in the limestone soil ($p > 0.05$) (Table 2). The N and P concentrations of the mixtures in yellow loam soil were similar to those in limestone soil (Table 2; Figure 2A,B). Similarly, Mo treatment had no significant effect on the N and P concentrations of ryegrass and white clover in either soil (Figure S2). However, in limestone soil, the Mo treatment had a significant effect on the N and P uptake of the mixtures ($p < 0.05$) (Table 2; Figure 2C,D). Specifically, when compared to the control in limestone soil, a 10 mg Mo kg^{-1} addition significantly increased plant N and P uptake ($p < 0.05$), while a 20 mg Mo kg^{-1} addition had no significant effect on the uptake of these elements ($p > 0.05$). Furthermore, the effects of the Mo addition on the N uptake of each forage type were similar: both increased with a 10 mg Mo kg^{-1} addition but demonstrated no significant effects with further Mo additions (Figure S3A,C). Regarding the P uptake of each forage in the limestone soil, the addition of 10 mg Mo kg^{-1} had no significant effect ($p > 0.05$), whereas it significantly decreased when 20 mg Mo kg^{-1} was added to the white clover ($p < 0.05$) (Figure S3B,D).

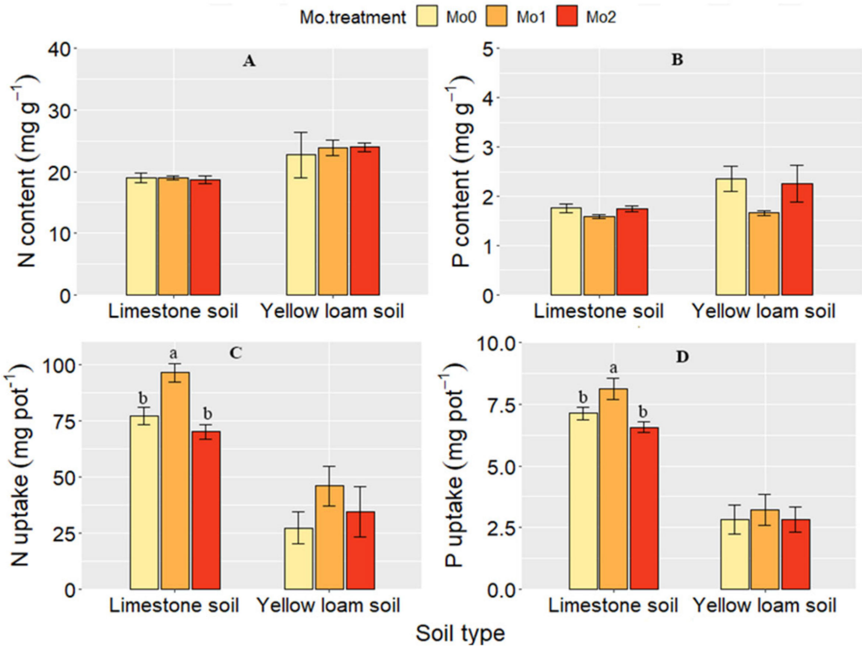

Figure 2. Nitrogen(N) and Phosphorus(P) (**A**,**B**) contents and N and P (**C**,**D**) uptake in shoots of mixtures (white clover + perennial ryegrass) under different Mo additions (Mo0, Mo1, and Mo2 represent 0, 10, and 20 mg Mo kg^{-1} soil in the form of Na_2MoO_4, respectively) to limestone soil and yellow loam soil. Different lowercase letters represent multiple comparisons of means among treatments determined by Duncan-test ($p < 0.05$).

3.3. Effects of Mo on Symbiotic Microorganisms and Its Link with Plant Growth

For perennial ryegrass, Mo addition had no significant effect on the AMF colonization in either soil ($p > 0.05$), whereas a high Mo addition significantly reduced the AMF colonization of the white clover in the yellow loam soil ($p < 0.05$) (Figure 3A,B). Mo addition had a significant impact on the number and weight of white clover nodules in limestone soil ($p < 0.05$) when compared to the yellow loam soil (Figure 3C,D). A 10 mg Mo kg^{-1} addition significantly increased the number and weight of nodules ($p < 0.05$), reaching the maximum value (103 nodules and 0.026 g, respectively). However, when compared to the 10 mg Mo kg^{-1} addition, the 20 mg Mo kg^{-1} addition significantly reduced the number and weight of the nodules. Pearson correlation coefficients revealed no significant relationships between the shoot biomass of the mixtures and the AMF colonization. In contrast, the shoot biomass of the mixture was only positively correlated with the number and weight of nodules in the limestone soil (Figure 4C,D).

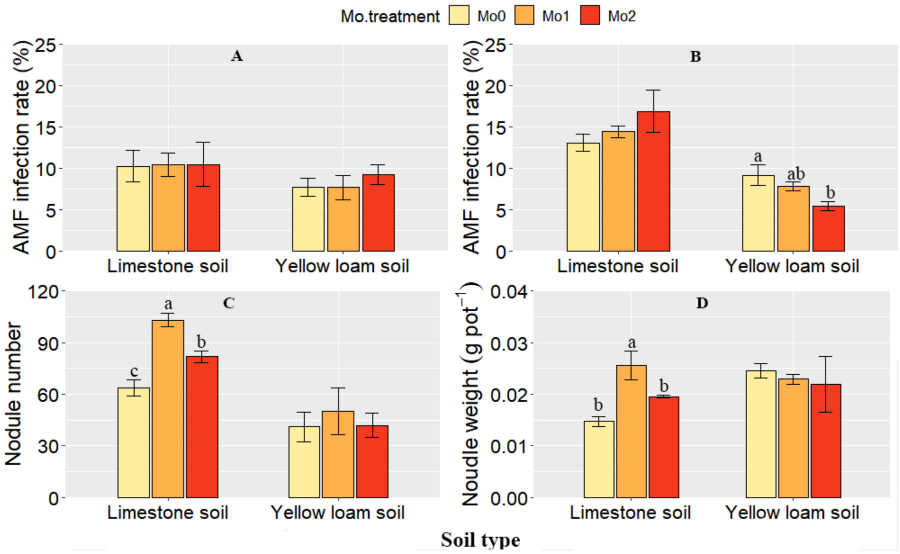

Figure 3. Effect of molybdenum additions (Mo0, Mo1, and Mo2 represent 0, 10, and 20 mg Mo kg^{-1} soil in the form of Na_2MoO_4, respectively) to limestone soil and yellow loam soil on perennial ryegrass (**A**) and white clover (**B**); the infection rate of AMF and nodule number (**C**) and nodule weight (**D**) in white clover roots. Different lowercase letters represent multiple comparisons of means among treatments determined by Duncan-test. ($p < 0.05$).

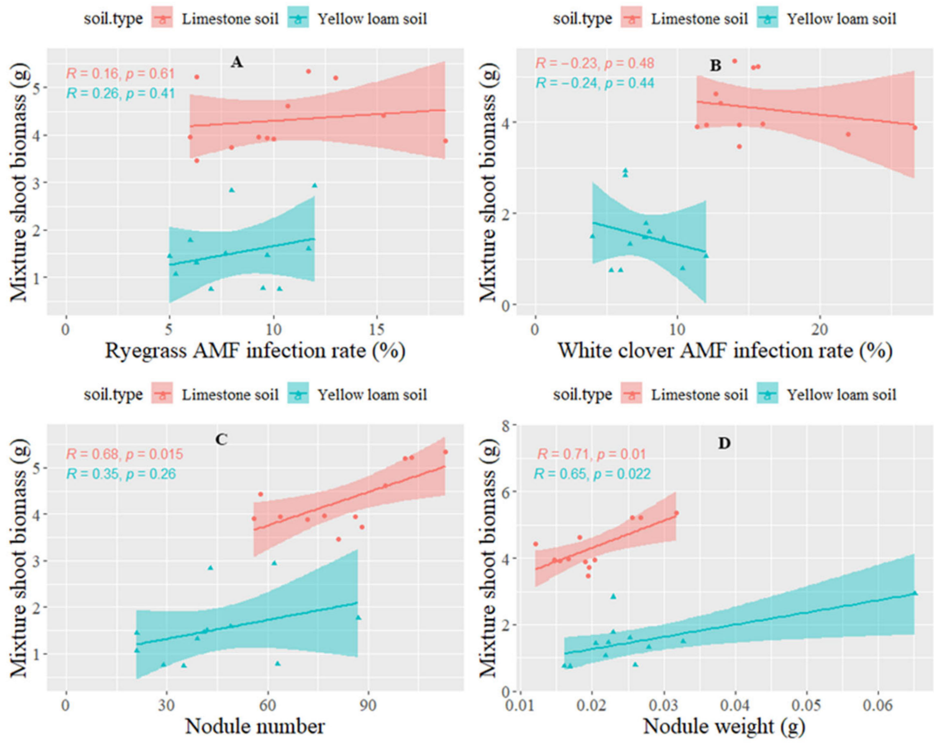

Figure 4. The correlations between AMF colonization of perennial ryegrass (**A**), AMF colonization of white clover (**B**), nodule number (**C**), and nodule weight (**D**) in white clover and shoot biomass of mixtures (white clover and perennial ryegrass).

4. Discussion

4.1. Influences of Mo Fertilizer on Plant Community Productivity

Forage yield improvement is often attributed to an increase in available nutrients [29,30]. Our findings indicate that Mo addition increased the biomass of ryegrass and white clover mixtures in limestone soils but not in yellow loam soils. These results are consistent with previous research [31–33]. However, a few studies found no significant differences in Mo fertilizer treatment on an aboveground biomass [34]. These inconsistencies may be due to differences in soil pH and available N (NH_4-N, NO_3-N). Low available N can directly inhibit the growth of ryegrass, which is a nitrophile [35]. A low soil pH also limits rhizobia activity and slows plant growth [36,37]. Adhikari and Missaoui [18] reported similar findings, stating that a low pH inhibits plant growth and root development.

The mechanism underlying the beneficial effect of Mo addition on biomass is primarily due to the promotion of root nodule growth caused by Mo addition, which helps improve plant uptake of N. This is because Mo, as a micronutrient, is an important part of nitrogenase, and exogenous Mo addition enhances the activity of nitrogenase, which in turn has a positive effect on root nodule production [38]. This interpretation was supported by the significant positive relationship between the shoot biomass and the number of root nodules in limestone soils (Figure 4C,D). However, the positive effects of the moderate Mo addition on the plant growth were not observed under treatments of high Mo addition. Similar results were reached in previous studies, which discovered that excess Mo is toxic to plants, inhibits plant growth, and reduces the amount of dry matter in the root system and aboveground parts [15,16]. Excess Mo fertilization also inhibits rhizobia, as is reflected by a decrease in the number and weight of symbiotic rhizobia. More research is needed to determine how excess Mo reduces symbiotic rhizobia.

4.2. Effects of Mo on the Main Nutrients of Forage

The N and P concentrations of forage are key indicators of forage nutrition, as these nutrients are vital for the production of milk and muscles. In the grasslands, livestock naturally select plants with high N or P concentrations [39]. An increased nutrient uptake can help increase nutrient concentrations in crop yields [40]. Several previous studies found that Mo supplementation increased the N and P content in plants by regulating the activities and expression of N-assimilating enzymes and inducing changes in the dynamics of P fractions in rhizosphere soil [41–43]. However, in our study, we found that Mo fertilization had no effect on the N and P concentrations of either species or their mixtures. These inconsistencies may be due to the different plant parts being measured; the leaf and stem have different functions (such as the leaf being the main metabolic organ), each of which responds differently to Mo fertilization [11,44]. Earlier studies have primarily focused on the leaves, which are sensitive to nutrient changes [31,33], whereas this study focused on the plant shoots. Stoichiometric homeostasis is another potential explanation for the negligible influence of Mo addition [45]; plants can maintain homeostasis in changing environments (e.g., soil nutrients) [46]. These results imply that both species can uptake more nutrients to maintain stoichiometric homeostasis as their biomass increases to avoid dilution effects.

5. Conclusions

In this study, we found that a 10 mg Mo kg^{-1} addition increased plant growth in mixed grasslands, but excess Mo addition demonstrated no further effects caused by reducing symbiotic rhizobia. The effects of Mo addition are also affected by the soil type, with significant effects demonstrated in limestone soil. Our results suggest that Mo fertilization can reduce N fertilization due to activation-enhanced, plant-symbiotic rhizobia, and it can also improve the N use efficiency. In terms of the implications for pasture practices, we recommend that N and P fertilization should be combined with Mo fertilizer to weaken environmental problems caused by fertilizer. Our research has potential implications for the management of artificial grasslands and improving grassland productivity. Finally, we highlight the need to the verify the extent to which the findings in a pot experiment can be found in a field.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13020495/s1, Figure S1: Shoot biomass in perennial ryegrass and white clover; Figure S2: N and P contents in perennial ryegrass and white clover; Figure S3: N and P uptake in perennial ryegrass and white clover.

Author Contributions: Conceptualization, J.C. and C.C.; formal analysis, J.Z.; investigation, J.Z.; data curation, J.Z.; writing—original draft preparation, J.Z.; writing—review and editing, J.C. and X.S.; visualization, J.Z.; supervision, J.C.; project administration, J.C.; funding acquisition, J.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the Guizhou Provincial Science and Technology Projects ([Qian Ke He [2022] Yi Ban040]; QKHPTRC-CXTD [2022] 011), the Guizhou Education Cooperation (Qianjiaoji [2022] 120), and the Open Foundation of Guizhou University Laboratory (SYSKF [2022] 029).

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated and analyzed during the current study are available from the authors upon reasonable request.

Acknowledgments: We thank Lin Li and Zongjie Liu for their contributions in sampling assistance. It was very helpful to receive suggestions and comments from anonymous reviewers.

Conflicts 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.

References

1. Huang, X.Y.; Hu, D.W.; Zhao, F.J. Molybdenum: More than an essential element. *J. Exp. Bot.* **2022**, *73*, 1766–1774. [CrossRef]
2. Imran, M.; Hu, C.; Hussain, S.; Rana, M.S.; Riaz, M.; Afzal, J.; Aziz, O.; Elyamine, A.M.; Ismael, M.A.F.; Sun, X. Molybdenum-induced effects on photosynthetic efficacy of winter wheat (*Triticum aestivum* L.) under different nitrogen sources are associated with nitrogen assimilation. *Plant Physiol. Biochem.* **2019**, *141*, 154–163. [CrossRef]
3. Bittner, F. Molybdenum metabolism in plants and crosstalk to iron. *Front. Plant Sci.* **2014**, *5*, 28. [CrossRef] [PubMed]
4. Barron, A.R.; Wurzburger, N.; Bellenger, J.P.; Wright, S.J.; Kraepiel, A.M.; Hedin, L.O. Molybdenum limitation of asymbiotic nitrogen fixation in tropical forest soils. *Nat. Geosci.* **2009**, *2*, 42–45. [CrossRef]
5. Valenciano, J.; Boto, J.; Marcelo, V. Chickpea (*Cicer arietinum* L.) response to zinc, boron and molybdenum application under field conditions. *N. Z. J. Crop Hortic. Sci.* **2011**, *39*, 217–229. [CrossRef]
6. Zou, C.; Gao, X.; Shi, R.; Fan, X.; Zhang, F. Micronutrient Deficiencies in Crop Production in China. In *Micronutrient Deficiencies in Global Crop Production*; Alloway, B.J., Ed.; Springer: Dordrecht, The Netherlands, 2008; pp. 127–148. [CrossRef]
7. Anke, M.; Seifert, M. The biological and toxicological importance of molybdenum in the environment and in the nutrition of plants, animals and man. Part 1: Molybdenum in plants. *Acta Biol. Hung.* **2007**, *58*, 311–324. [CrossRef]
8. Ankerman, D.; Large, R. *Soil and Plant Analysis*; A&L Agricultural Laboratories, Inc.: New York, NY, USA, 1974.
9. Chatterjee, C.; Nautiyal, N. Molybdenum Stress Affects Viability and Vigor of Wheat Seeds. *J. Plant Nutr.* **2006**, *24*, 1377–1386. [CrossRef]
10. Majda, C.; Khalid, D.; Aziz, A.; Rachid, B.; Badr, A.S.; Lotfi, A.; Mohamed, B. Nutri-priming as an efficient means to improve the agronomic performance of molybdenum in common bean (*Phaseolus vulgaris* L.). *Sci. Total Environ.* **2019**, *661*, 654–663. [CrossRef]
11. Liu, L.; Xiao, W.; Li, L.; Li, D.-M.; Gao, D.-S.; Zhu, C.-Y.; Fu, X.-L. Effect of exogenously applied molybdenum on its absorption and nitrate metabolism in strawberry seedlings. *Plant Physiol. Biochem.* **2017**, *115*, 200–211. [CrossRef] [PubMed]
12. Inostroza, L.; Bhakta, M.; Acuña, H.; Vásquez, C.; Ibáñez, J.; Tapia, G.; Mei, W.; Kirst, M.; Resende, M., Jr.; Munoz, P. Understanding the complexity of cold tolerance in white clover using temperature gradient locations and a GWAS approach. *Plant Genome* **2018**, *11*, 170096. [CrossRef]
13. García-Favre, J.; López, I.F.; Cranston, L.M.; Donaghy, D.J.; Kemp, P.D.; Ordóñez, I.P. Functional contribution of two perennial grasses to enhance pasture production and drought resistance under a leaf regrowth stage defoliation criterion. *J. Agron. Crop Sci.* **2022**, *209*, 144–160. [CrossRef]
14. Banerjee, P.; Nath, R. Prospects of molybdenum fertilization in grain legumes—A review. *J. Plant Nutr.* **2022**, *45*, 1425–1440. [CrossRef]
15. Gopal, R. Effect of molybdenum stress on growth, yield and seed quality in black gram. *J. Plant Nutr.* **2016**, *39*, 463–469. [CrossRef]
16. Mcgrath, S.P.; Mico, C.; Zhao, F.J.; Stroud, J.L.; Zhang, H.; Fozard, S. Predicting molybdenum toxicity to higher plants: Estimation of toxicity threshold values. *Environ. Pollut.* **2010**, *158*, 3085–3094. [CrossRef]
17. Gupta, U.C. Boron and molybdenum nutrition of wheat, barley and oats grown in Prince Edward Island soils. *Can. J. Soil Sci.* **1971**, *51*, 415–422. [CrossRef]

18. Adhikari, L.; Missaoui, A.M. Nodulation response to molybdenum supplementation in alfalfa and its correlation with root and shoot growth in low pH soil. *J. Plant Nutr.* **2017**, *40*, 2290–2302. [CrossRef]
19. Unathi, G.; Nobulungisa, M.; Solomon, T.B. Benefits of grass-legume inter-cropping in livestock systems. *Afr. J. Agric. Res.* **2018**, *13*, 1311–1319. [CrossRef]
20. Van Der Heijden, M.G.; Bakker, R.; Verwaal, J.; Scheublin, T.R.; Rutten, M.; Van Logtestijn, R.; Staehelin, C. Symbiotic bacteria as a determinant of plant community structure and plant productivity in dune grassland. *FEMS Microbiol. Ecol.* **2006**, *56*, 178–187. [CrossRef] [PubMed]
21. Zhou, J.; Wilson, G.W.; Cobb, A.B.; Zhang, Y.; Liu, L.; Zhang, X.; Sun, F. Mycorrhizal and rhizobial interactions influence model grassland plant community structure and productivity. *Mycorrhiza* **2022**, *32*, 15–32. [CrossRef]
22. Alam, F.; Kim, T.Y.; Kim, S.Y.; Alam, S.S.; Pramanik, P.; Kim, P.J.; Lee, Y.B. Effect of molybdenum on nodulation, plant yield and nitrogen uptake in hairy vetch (*Vicia villosa* Roth). *Soil Sci. Plant Nutr.* **2015**, *61*, 664–675. [CrossRef]
23. Hafner, H.; Ndunguru, B.; Bationo, A.; Marschner, H. Effect of nitrogen, phosphorus and molybdenum application on growth and symbiotic N2-fixation of groundnut in an acid sandy soil in Niger. *Fertil. Res.* **1992**, *31*, 69–77. [CrossRef]
24. Battini, F.; Gronlund, M.; Agnolucci, M.; Giovannetti, M.; Jakobsen, I. Facilitation of phosphorus uptake in maize plants by mycorrhizosphere bacteria. *Sci. Rep.* **2017**, *7*, 4686. [CrossRef]
25. Shi, Z.; Zhang, J.; Lu, S.; Li, Y.; Wang, F. Arbuscular mycorrhizal fungi improve the performance of sweet sorghum grown in a mo-contaminated soil. *J. Fungi* **2020**, *6*, 44. [CrossRef] [PubMed]
26. Piao, H.; Wu, Y.; Hong, Y.; Yuan, Z. Soil-released carbon dioxide from microbial biomass carbon in the cultivated soils of karst areas of southwest China. *Biol. Fertil. Soils* **2000**, *31*, 422–426. [CrossRef]
27. Mei, L.; Yang, X.; Zhang, S.; Zhang, T.; Guo, J. Arbuscular mycorrhizal fungi alleviate phosphorus limitation by reducing plant N:P ratios under warming and nitrogen addition in a temperate meadow ecosystem. *Sci. Total Environ.* **2019**, *686*, 1129–1139. [CrossRef] [PubMed]
28. Tao, Y.; Qiu, D.; Gong, Y.-M.; Liu, H.-L.; Zhang, J.; Yin, B.-F.; Lu, H.-Y.; Zhou, X.-B.B.; Zhang, Y.-M. Leaf-root-soil N:P stoichiometry of ephemeral plants in a temperate desert in Central Asia. *J. Plant Res.* **2022**, *135*, 55–67. [CrossRef] [PubMed]
29. Delevatti, L.M.; Cardoso, A.S.; Barbero, R.P.; Leite, R.G.; Reis, R.A. Effect of nitrogen application rate on yield, forage quality, and animal performance in a tropical pasture. *Sci. Rep.* **2019**, *9*, 7596. [CrossRef] [PubMed]
30. Qiao, L.; Wang, X.; Smith, P.; Fan, J.; Lu, Y.; Emmett, B.; Li, R.; Dorling, S.; Chen, H.; Liu, S.; et al. Soil quality both increases crop production and improves resilience to climate change. *Nat. Clim. Change* **2022**, *12*, 574–580. [CrossRef]
31. Rana, M.S.; Hu, C.X.; Shaaban, M.; Imran, M.; Afzal, J.; Moussa, M.G.; Elyamine, A.M.; Bhantana, P.; Saleem, M.H.; Syaifudin, M.; et al. Soil phosphorus transformation characteristics in response to molybdenum supply in leguminous crops. *J. Environ. Manag.* **2020**, *268*, 110610. [CrossRef]
32. Babenko, O.N.; Brychkova, G.; Sagi, M.; Alikulov, Z.A. Molybdenum application enhances adaptation of crested wheatgrass to salinity stress. *Acta Physiol. Plant.* **2015**, *37*, 14. [CrossRef]
33. Rana, M.S.; Sun, X.; Imran, M.; Ali, S.; Shaaban, M.; Moussa, M.G.; Khan, Z.; Afzal, J.; Binyamin, R.; Bhantana, P. Molybdenum-induced effects on leaf ultra-structure and rhizosphere phosphorus transformation in *Triticum aestivum* L. *Plant Physiol. Biochem.* **2020**, *153*, 20–29. [CrossRef] [PubMed]
34. Ma, J.; Bei, Q.; Wang, X.; Lan, P.; Liu, G.; Lin, X.; Liu, Q.; Lin, Z.; Liu, B.; Zhang, Y.; et al. Impacts of Mo application on biological nitrogen fixation and diazotrophic communities in a flooded rice-soil system. *Sci. Total Environ.* **2019**, *649*, 686–694. [CrossRef] [PubMed]
35. Jossi, M.; Fromin, N.; Tarnawski, S.; Kohler, F.; Gillet, F.; Aragno, M.; Hamelin, J. How elevated pCO_2 modifies total and metabolically active bacterial communities in the rhizosphere of two perennial grasses grown under field conditions. *FEMS Microbiol. Ecol.* **2006**, *55*, 339–350. [CrossRef] [PubMed]
36. Alemayehu, D.; Zerihun, A.; Solomon, B. Limitations and strategies to enhance biological nitrogen fixation in sub-humid tropics of Western Ethiopia. *J. Agric. Biotechnol. Sustain. Dev.* **2018**, *10*, 122–131. [CrossRef]
37. Jaiswal, S.K.; Naamala, J.; Dakora, F.D. Nature and mechanisms of aluminium toxicity, tolerance and amelioration in symbiotic legumes and rhizobia. *Biol. Fertil. Soils* **2018**, *54*, 309–318. [CrossRef]
38. Rana, M.S.; Bhantana, P.; Imran, M.; Saleem, M.H.; Moussa, M.G.; Khan, Z.; Khan, I.; Alam, M.; Abbas, M.; Binyamin, R. Molybdenum potential vital role in plants metabolism for optimizing the growth and development. *Ann. Environ. Sci. Toxicol.* **2020**, *4*, 32–44.
39. Pauler, C.M.; Isselstein, J.; Suter, M.; Berard, J.; Braunbeck, T.; Schneider, M.K. Choosy grazers: Influence of plant traits on forage selection by three cattle breeds. *Funct. Ecol.* **2020**, *34*, 980–992. [CrossRef]
40. Li, W.X.; Lu, J.W.; Seneweera, S.P.; Chen, F.; Lu, J.M.; Li, X.K. Effect of fertilization on forage yield and quality, nutrients uptake and soil properties in the more intensive cropping system. *J. Food Agric. Environ.* **2010**, *8*, 427–434. [CrossRef]
41. Imran, M.; Sun, X.; Hussain, S.; Rana, M.S.; Saleem, M.H.; Riaz, M.; Tang, X.; Khan, I.; Hu, C. Molybdenum supply increases root system growth of winter wheat by enhancing nitric oxide accumulation and expression of NRT genes. *Plant Soil* **2021**, *459*, 235–248. [CrossRef]
42. Qin, S.; Hu, C.; Tan, Q.; Sun, X. Effect of molybdenum levels on photosynthetic characteristics, yield and seed quality of two oilseed rape (*Brassica napus* L.) cultivars. *Soil Sci. Plant Nutr.* **2017**, *63*, 137–144. [CrossRef]

43. Sabatino, L.; D'Anna, F.; Iapichino, G.; Moncada, A.; D'Anna, E.; De Pasquale, C. Interactive Effects of Genotype and Molybdenum Supply on Yield and Overall Fruit Quality of Tomato. *Front. Plant Sci.* **2019**, *9*, 01922. [CrossRef] [PubMed]
44. Liu, H.; Hu, C.; Hu, X.; Nie, Z.; Sun, X.; Tan, Q.; Hu, H. Interaction of molybdenum and phosphorus supply on uptake and translocation of phosphorus and molybdenum by *Brassica napus*. *J. Plant Nutr.* **2010**, *33*, 1751–1760. [CrossRef]
45. Persson, J.; Fink, P.; Goto, A.; Hood, J.M.; Jonas, J.; Kato, S. To be or not to be what you eat: Regulation of stoichiometric homeostasis among autotrophs and heterotrophs. *Oikos* **2010**, *119*, 741–751. [CrossRef]
46. Yu, Q.; Wilcox, K.; Pierre, K.L.; Knapp, A.K.; Han, X.; Smith, M.D. Stoichiometric homeostasis predicts plant species dominance, temporal stability, and responses to global change. *Ecology* **2015**, *96*, 2328–2335. [CrossRef] [PubMed]