

Magnesium Fertilizer-Induced Increase of Symbiotic Microorganisms Improves Forage Growth and Quality

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S Supporting Information

ABSTRACT: Magnesium (Mg) plays important roles in photosynthesis and protein synthesis; however, latent Mg deficiencies are common phenomena that can influence food quality. Nevertheless, the effects of Mg fertilizer additions on plant carbon (C):nitrogen (N):phosphorus (P) stoichiometry, an important index of food quality, are unclear and the underlying mechanisms unexplored. We conducted a greenhouse experiment using low-Mg in situ soil without and with a gradient of Mg additions to investigate the effect of Mg fertilizer on growth and stoichiometry of maize and soybean and also measure these plants' main symbiotic microorganisms: arbuscular mycorrhizal fungi (AMF) and rhizobium, respectively. Our results showed that Mg addition significantly improved both plant species' growth and also increased N and P concentrations in soybean and maize, respectively, resulting in low C:N ratio and high N:P ratio in soybean and low C:P and N:P ratios in maize. These results presumably stemmed from the increase of nutrients supplied by activation-enhanced plant symbiotic microorganisms, an explanation supported by statistically significant positive correlations between plant stoichiometry and plants' symbiotic microorganisms' increased growth with Mg addition. We conclude that Mg supply can improve plant growth and alter plant stoichiometry via enhanced activity of plant symbiotic microorganisms. Possible mechanisms underlying this positive plant–soil feedback include an enhanced photosynthetic product flow to roots caused by adequate Mg supply.

KEYWORDS: magnesium, plant symbiotic microorganisms, plant growth and stoichiometry, maize, soybean

■ INTRODUCTION

Magnesium (Mg) is essential for the growth and development of plants and plays an important role in photosynthesis because it is the central element of chlorophyll and is a cofactor of a series of enzymes involved in carbon fixation. Mg also plays a fundamental role in phloem export of photosynthates from source to sink organs such as roots.¹ Latent and acute Mg deficiencies in plants are common phenomena because of its unique chemical property—a highly hydrated radius that sorbs less to colloids than other cations.² Magnesium fertilizer not only increases plant photosynthesis by mitigating Mg deficiencies^{3–7} but may also improve other nutrients' uptake by roots via enhancing transport of photosynthetic products to roots. Therefore, the physiological and biochemical changes caused by Mg fertilizer can not only increase plant growth but may also alter plant stoichiometry, which is a key characteristic trait that controls nutrients and energy transfer in food webs.^{8,9} Understanding the influence of Mg fertilizer on plant stoichiometry should thus provide important new information about physiological and ecological ramifications of mineral nutrients in terrestrial ecosystems. To our knowledge, no study has researched the influences of Mg fertilizer on plant stoichiometry, although such measurements can be very important for predicting plant-to-herbivore energy and nutrient flow.

Ecological stoichiometry considers that all organisms are composed of the same major elements [carbon (C), nitrogen (N), and phosphorus (P)]. The balance of these elements as

well as their supply affects consumers, and change of plant food stoichiometry measurements can provide perspective to predict the possible effects of fertilizer or other treatments on consumer animal growth.¹⁰ Studies of autotroph–herbivore interface (particularly for freshwater ecosystems) show that N or P fertilizer treatment changes plant C:N:P stoichiometry, which then influences energy and mineral flow in these ecosystems.^{11–13} These studies found that food (plant) with moderate C:P and N:P ratios are most likely to result in efficient element trophic transfer, as excessively low or high C:P and N:P food (plant) ratios are not beneficial to the growth of consumers (herbivores).^{11,13–15} Forage quality studies have generally focused on the contents of crude protein (a reflection of N content), although few studies have shown that Mg fertilizer enhanced protein content in food, but the underlying mechanism is unclear.⁷

Mg fertilizer may enhance N and P acquisition by plant root via improving root growth and perhaps by enhancement of symbiotic microorganisms, which deliver limiting soil minerals (e.g., N and P) to their host plants in return for photosynthetically derived carbon.^{16–20} There is strong empirical evidence that Mg fertilizer results in dramatic increases in carbohydrate flow to roots,^{1,3,5} resulting in both enhanced root growth and

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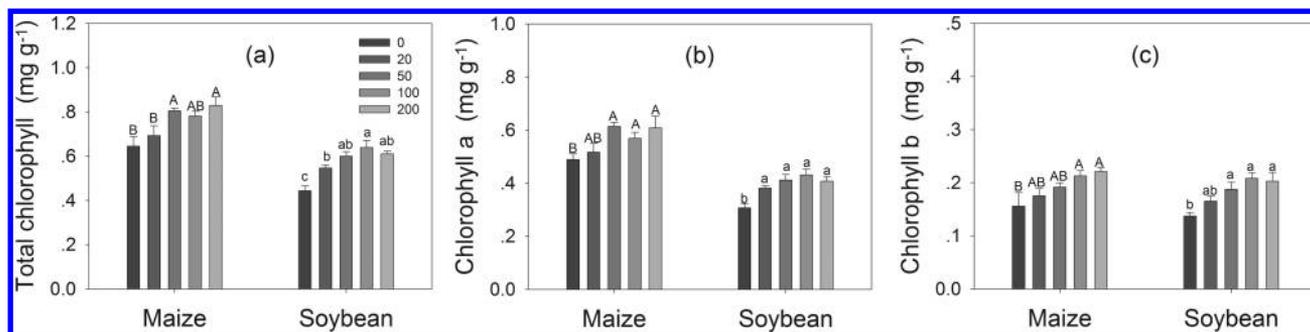


Figure 1. Effect of magnesium addition (20, 50, 100, and 200 mg kg⁻¹ MgCO₃) to acid red-yellow (low Mg) soil on total chlorophyll (a), chlorophyll a (b), and chlorophyll b (c) in maize and soybean leaves. Different letters (capital for maize and lower case for soybean) indicate significant differences between treatment means (Student–Newman–Keuls test, $p < 0.05$).

enlarged contact area of root with soil.^{1,21–23} Additionally, preferential partitioning of photosynthetic carbon to roots to increase limited elements' uptake has also been reported.^{21,22} This increase of root carbohydrates caused by Mg fertilizer might also enhance the mutualistic interactions between host plant and microbes such as arbuscular mycorrhizal fungi (AMF) in grasses and rhizobium in legumes. With Mg fertilizer, this increase of both root growth and plant mutualistic microorganisms could together enhance the nutrient and water acquisition by roots, perhaps altering plant growth as well as C:N:P stoichiometry.²³

In the current study, a greenhouse experiment was conducted to investigate the effects of Mg fertilizer in low-Mg soil on forage maize and soybean growth and stoichiometry. The influence of Mg fertilizer on colonization of these plants' mutualistic microorganisms was also evaluated. We hypothesized that (i) Mg addition in both species would improve plant growth and activate plant mutualistic microorganisms; (ii) Mg addition would enhance N, P, and Mg concentrations in leaves (resulting in decreased C:P and C:N ratios) in both species; and (iii) Mg addition would bring no change in N:P ratio in maize (a grass) but would increase N:P ratio in soybean (a legume) due to different properties of arbuscular mycorrhizal fungi and rhizobium.

MATERIALS AND METHODS

Experimental Design. Acid red-yellow soil in southern China is usually poor in Mg because of soil acidification and leaching due to high precipitation, and plants in this soil also suffer from P deficiency. Therefore, we collected the acid red-yellow soil from Hengyang Red Soil Experimental Station in Hunan province and mixed for homogeneity. The five mixed soil samples were air-dried in a shaded and ventilated environment for approximately 1 month to achieve a constant weight for determination of soil pH and total elements. The soil total N, P, available Mg, and organic carbon concentrations and pH were 0.082%, 0.036%, 0.0015%, 1.33%, and 4.3, respectively.

A total of 50 plastic pots (20 cm in diameter, 24 cm in depth) were filled with the well-mixed soil, each pot containing 4 kg soil. The soil-filled pots were then divided into two groups of 25 pots per group, resulting in 25 pots for each of the two species (maize or soybean). As done on previous studies conducted at this site, we set five treatments of Mg addition (0, 20, 50, 100, and 200 mg kg⁻¹ MgCO₃) for each group (one species) with five replications. The total MgCO₃ fertilizer (powder) was calculated according to each treatment concentration and soil weight.

Plant Materials. Maize and soybean were selected as model species for close relationships with AMF and rhizobium, respectively. At the beginning of May, ~10 maize or soybean seeds were sown in each pot in the greenhouse. We watered the seedlings every other day, and the water level was adjusted using an electronic weigher once

every 3 days to maintain similar soil moisture contents. The pot position was also changed randomly once every 3 days to avoid light heterogeneity. After 3 weeks, the seedlings (~10 cm) were thinned in each pot to allow sufficient space for seedling growth with three similar seedlings kept in each pot. We gently removed the upper soil (~2 cm) from each pot, then evenly sprinkled corresponding MgCO₃, and replaced the previously removed soil. After fertilization, we evenly sprinkled the surface with water to help plant take up the fertilizer.

Sampling and Chemical Analysis. After 8 weeks, we harvested the plants. All plants were divided into roots and shoots. The roots were cleaned with tap water and then ultrapure water and finally blotted dry with superabsorbent paper. One-tenth of fresh roots of maize were used for measuring AMF colonization (see below). The root nodules of soybean were removed, counted, and weighed using a ten-thousandth analytical electronic balance after they had been blotted dry with superabsorbent paper. These weights and numbers of soybean nodules were used to quantify the degree of rhizobium colonization. To measure chlorophyll content, about 2 g mature fresh leaves was extracted with 95(v/v) ethanol, and chlorophyll was measured using spectrophotometry.²⁴ Remaining parts of plant samples and nodules were oven-dried at 65 °C for 72 h. The nodules, shoots, and roots (without rhizobium nodules) were cooled to room temperature in a desiccator and were weighed to determine dry biomass. Leaves and stems were then separated for each shoot; only mature dried leaves were used for elemental analysis and stoichiometry calculations. In preparation for chemical analyses, all plant samples were ground in 20-in. mesh in a Wiley mill. All samples were kept cool and dry for chemical analysis.

AMF Colonization. The roots used to measure AMF colonization were cut into approximately 1 cm lengths and were cleared in 10% (w/v) KOH at 90 °C in a water bath for 30 min, then washed and stained with 0.05% (w/v) Trypan blue developed by Trouvelet.²⁵ Using a dissection microscope, 30 root segments of each sample were assessed for percentage of root length colonized by AMF.

Elemental Analyses of Leaf Samples. Dried mature leaf samples were analyzed for total C and N concentrations (mg g⁻¹) utilizing an elemental analyzer (Vario EL III, Elementar, Germany) and for P and Mg using an inductively coupled plasma emission spectrometer (Iris Advantage 1000, USA) once the samples were digested using trace-metal-grade nitric and perchloric acid, and diluted in 100 mL of double-distilled water.

Statistical Analysis. The data were log-transformed when necessary to meet the parametric test assumptions of normality (Bartlett test) and homogeneous variances, and then one-way ANOVA was used to determine the effects of Mg treatment on plant growth, leaf stoichiometry, and colonization rate of AMF and rhizobia. If data after transformation did not meet the parametric test assumptions of normality, the Kruskal–Wallis test was used. Pearson's product-moment correlation was used to analyze the relationships among plant growth, leaf stoichiometry, and plant symbiotic microorganisms. Data analyses were performed with R 3.0.2 (R Development Core Team 2005).

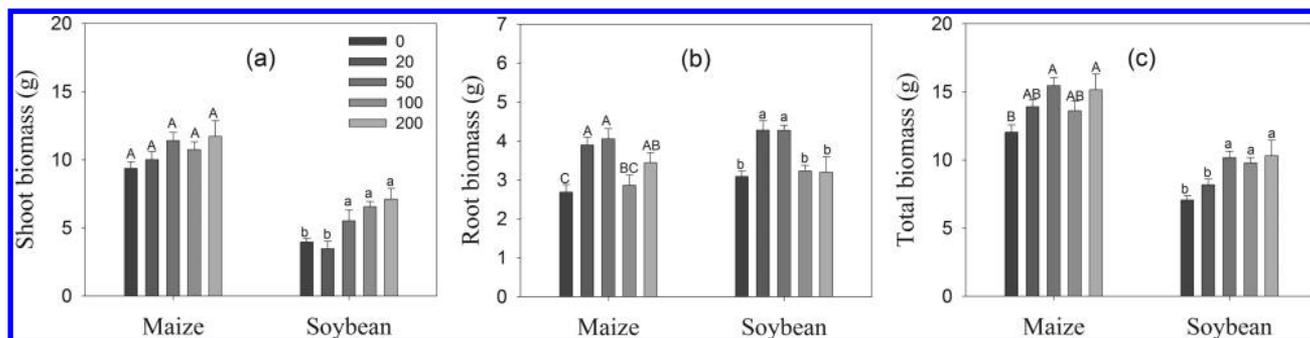


Figure 2. Influence of magnesium additions (20, 50, 100, and 200 mg kg⁻¹ MgCO₃) to acid red-yellow (low Mg) soil on shoot (a) and root biomass (b) and total biomass (c) in maize and soybean. Different letters (capital for maize and lower case for soybean) indicate significant differences between treatment means (Student–Newman–Keuls test, $p < 0.05$).

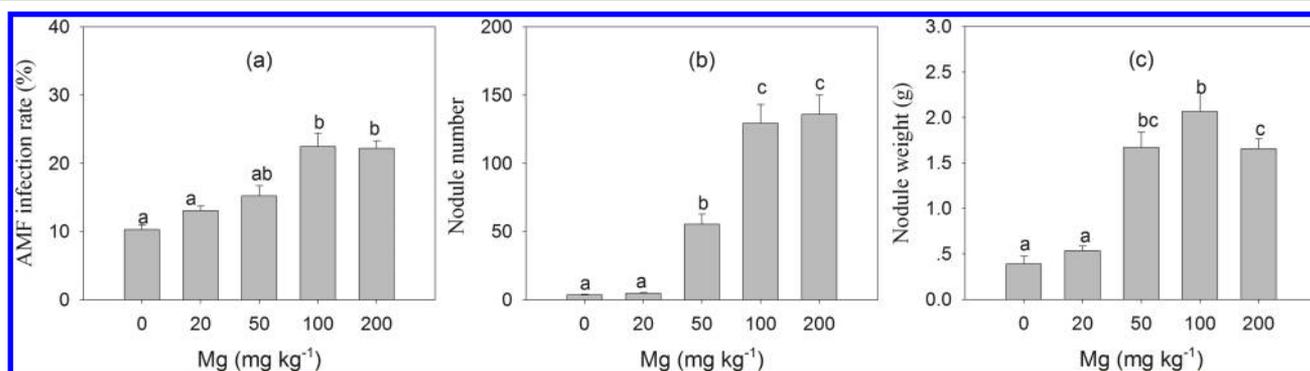


Figure 3. Effect of magnesium additions (20, 50, 100, and 200 mg kg⁻¹ MgCO₃) to acid red-yellow soil on infection rate of AMF (a) in maize roots and (b, c) in rhizobium in soybean. Different letters indicate significant differences between treatment means (Student–Newman–Keuls test, $p < 0.05$).

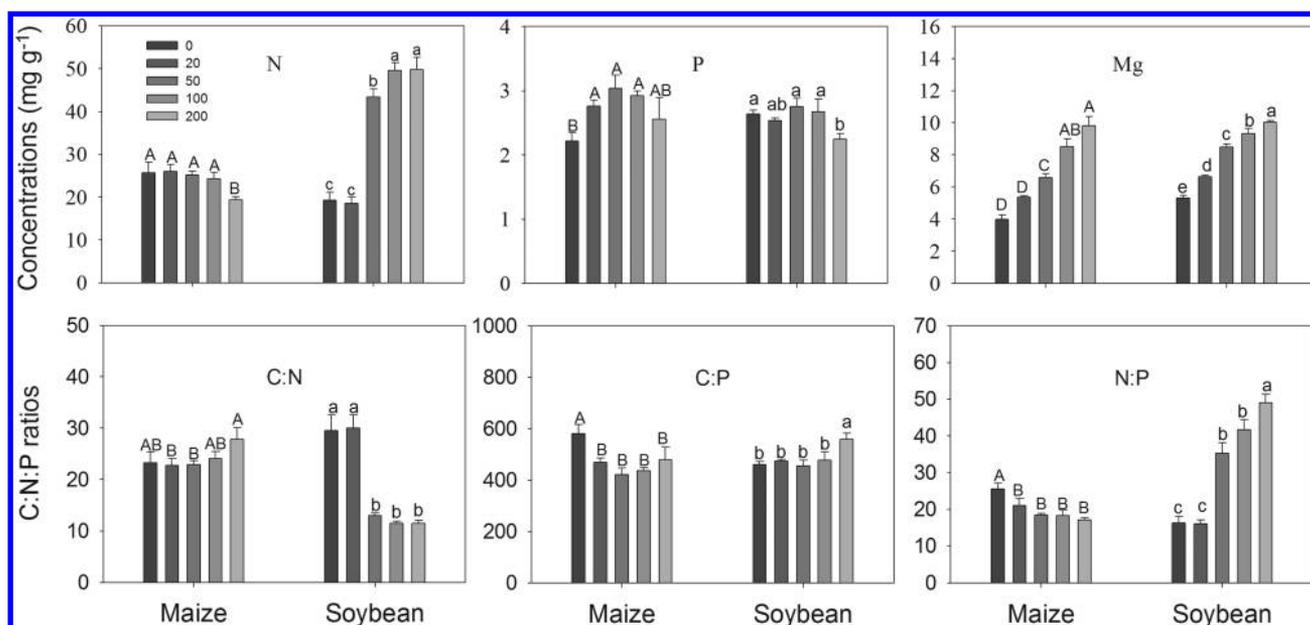


Figure 4. Nitrogen (N), phosphorus (P), and magnesium (Mg) concentrations and C:N:P ratios in leaves of maize and soybean under different magnesium additions (20, 50, 100, and 200 mg kg⁻¹ MgCO₃) to acid-red-yellow soil. Different letters (capital for maize and lower case for soybean) indicate significant differences between treatment means (Student–Newman–Keuls test, $p < 0.05$).

RESULTS

Effects of Magnesium Fertilizer on Leaf Chlorophyll and Plant Growth. Total chlorophyll, chlorophyll *a*, and chlorophyll *b* concentrations in mature leaves were significantly increased by Mg addition in both species, at >50 mg kg⁻¹

addition level in maize and >20 mg kg⁻¹ Mg addition for soybean (Figure 1). Mg fertilizer showed no significant influence on shoot biomass of maize, but significantly increased the shoot biomass of soybean (Figure 2a). The root biomass of both species increased with low Mg additions (20 or 50 mg

Table 1. Results of Soybean Relationships (Pearson's Product-Moment Correlation) among Shoot Biomass (SB), Leaf Stoichiometry, and Its Symbiotic Microorganisms^a

characteristic	TNR	Chl	SB	N	P	C:N	C:P	N:P
TWR	0.89*	0.91*	0.88 [#]	0.98**	-0.0008	-0.98**	0.31	0.91*
TNR		0.82	0.98**	0.94*	-0.39	-0.9*	0.66	0.97**
Chl			0.87 [#]	0.86 [#]	-0.13	-0.86	0.38	0.83
SB				0.90	-0.41	-0.86	0.65	0.93*
N					-0.18	-0.995**	0.48	0.98**
P						0.11	-0.95*	-0.38
C:N							-0.42	-0.96**
C:P								0.65

^a* and ** represent significance at $\alpha = 0.05$, and 0.01 , respectively, and # indicates marginal significance ($0.06 > \alpha > 0.05$). TWR, average total weight of nodules/plant; TNR, average total number of rhizobium nodules/plant; Chl, chlorophyll; N, nitrogen; P, phosphorus; C, carbon.

Table 2. Results of Maize Relationships (Pearson's Product-Moment Correlation) among Shoot Biomass (SB), Leaf Stoichiometry, and Its Symbiotic Microorganisms^a

characteristic	Chl	SB	N	P	C:N	C:P	N:P
AMF	0.99**	0.83	-0.71	0.56	0.61	-0.71	-0.94*
Chl		0.89*	-0.69	0.61	0.60	-0.74	-0.96*
SB			-0.80	0.31	0.76	-0.46	-0.80
N				0.12	-0.99**	0.11	0.62
P					-0.22	-0.97**	-0.71
C:N						-0.01	-0.53
C:P							0.85

^a* and ** represent significance at $\alpha = 0.05$ and 0.01 , respectively. AMF, arbuscular mycorrhizal fungi; Chl, chlorophyll; N, nitrogen; P, phosphorus; C, carbon.

$\text{kg}^{-1} \text{MgCO}_3$), whereas high Mg addition ($100\text{--}200 \text{ mg kg}^{-1} \text{MgCO}_3$) showed no effects (Figure 2b). Low Mg addition ($20 \text{ mg kg}^{-1} \text{MgCO}_3$) showed no effects on total biomass, whereas moderate (50 or 100 mg kg^{-1}) Mg additions had significant positive effects on total biomass and high Mg additions had no further positive effect (Figure 2b,c).

Effects of Magnesium Fertilizer on Plant Mutualistic Microorganisms. Except for low Mg treatment levels (20 or $50 \text{ mg kg}^{-1} \text{MgCO}_3$), Mg treatment up to 100 mg kg^{-1} significantly increased AMF colonization in maize, and the effect of further additional Mg (at high Mg treatment 200 mg kg^{-1}) showed no further improved effect (Figure 3a). The rhizobium showed a pattern similar to that of AMF with Mg addition, reflected in both high nodulation and total nodule weight of each plant in response to Mg addition (Figure 3b,c).

Effects of Magnesium Fertilizer on Plant Stoichiometry. The dry leaf Mg concentration of both species significantly increased with Mg addition (Figure 4), but there was no significant change of C concentration (data not shown in Figure 4). Mg addition showed no significant effects on N concentration in maize leaf, except at the highest level of Mg addition, which significantly decreased N concentration. In contrast, all levels of Mg addition $>20 \text{ mg kg}^{-1}$ showed a significant increase of N in soybean leaf. Mg addition, except at the highest level (200 mg kg^{-1}), significantly increased P concentration in maize leaf, whereas there was no significant influence of Mg treatments on P in soybean leaf except at the highest level of Mg addition, which significantly decreased P concentration (Figure 4).

All levels of Mg addition reduced N:P ratio significantly in maize leaf (Figure 4), but Mg addition at levels $>20 \text{ mg g}^{-1}$ significantly enhanced N:P ratio in soybean leaf. Furthermore, the N:P ratio in maize leaf was reduced similarly at all levels of Mg addition ($20\text{--}200 \text{ mg kg}^{-1} \text{MgCO}_3$) (Figure 4). In

contrast, Mg addition of 50 and $100 \text{ mg kg}^{-1} \text{MgCO}_3$ doubled leaf N:P ratio, and $200 \text{ mg kg}^{-1} \text{Mg}$ addition further increased leaf N:P of soybean leaves significantly and substantially another 40% (Figure 4). The C:N ratio in soybean leaves significantly decreased with Mg addition except for $20 \text{ mg kg}^{-1} \text{MgCO}_3$, whereas there were no significant influences on C:N ratio in maize leaves (Figure 4). Mg addition decreased C:P ratio in maize leaves with no difference between treatments, but showed no influence on C:P ratio in soybean leaves except for a significant increase at the highest level of Mg addition (Figure 4).

Relationships of Plant Biomass or Stoichiometry with Symbiotic Microorganisms. The average weight and number of individual plant rhizobium nodules showed significant positive correlation with both plant biomass and mature leaf N concentration (resulting in negative relationships between weight of rhizobium and C:N ratio and positive relationships with N:P ratio) (Table 1). By contrast, the AMF colonization showed no significant relationships with plant biomass and measured elements in mature leaf, but did show a significant negative relationship with N:P ratio (Table 2).

DISCUSSION

Influences of Magnesium Fertilizer on Plant Growth. As in recent studies,^{6,7} appropriate Mg fertilizer significantly increased chlorophyll content in both maize and soybean, reflecting an increase of photosynthesis under Mg fertilizer conditions. Similar to some previously reported findings that Mg fertilizer increased plant biomass,^{1,6,7,26} we found that shoot, root, and total biomass increased in soybean with Mg addition, whereas in maize only root and total biomass increased. A few studies also have observed no effects of Mg fertilizer on biomass of shoot.^{6,27-29} These inconsistent results may be due to different physiological and biochemical

responses of plants and their symbiotic microorganisms to Mg addition.^{6,29,30} Positive effects of moderate Mg addition on root growth were not seen in either species under high Mg addition conditions. Perhaps this reflects allocation of more photosynthetic C to root mutualistic microorganisms rather than to root growth as a more efficient mode of nutrient acquisition. This interpretation was supported by high AMF colonization in maize and increased ratio of root nodule biomass:root biomass in soybean with Mg addition (Figure S1).

Colonization of Plant Mutualistic Microorganisms in Response to Magnesium Fertilizer. *Rhizobium.* As expected, Mg-treated soybean plants had more and larger nodules. Similar results were reported by Kiss et al.,²⁷ and they considered that Mg has an important role in the metabolism of the rhizobium bacteria and nodule development because (N₂)-fixing rhizobium strains need ATP that must be present as a Mg complex.²⁷ Another possible contributing mechanism for the positive influence of Mg addition on rhizobium bacteria may be the increase of carbon flow to root induced by Mg addition,¹ making more nutritional C available to rhizobium for growth. Although we did not find a significant increase of sugar in roots under Mg addition (except for low Mg addition) to support this interpretation, this may be because we measured the sugar in only the roots without nodules. This needs to be tested.

AMF. As found here, a previous study showed that Mg addition has a pronounced positive effect on root AMF infection of maize, a result that could not be explained by changes in pH or osmotic pressure of the nutrient solution.²⁸ Conversely, few other studies reported an inhibitory effect of Mg fertilizer on colonization of mycorrhiza fungi.^{31,32} These inconsistent results may arise from an imbalance of Mg and other elements, such as Ca, which can disrupt the mycorrhizal association with its host plant, or differences of bacterial strain.^{31,32} Here, we also suggest that the increase of mutualistic association was attributable to the enhanced phloem export of sucrose under Mg addition conditions (Figure S2).

Plant Stoichiometry in Response to Magnesium Fertilizer. *Nitrogen.* As our hypothesis predicted, Mg addition doubled N (or protein) concentration in soybean leaf, presumably as a result of the increase of biological nitrogen fixation in larger and more numerous root nodules. In contrast, there was no rise and even a slight decrease in maize leaf N concentration with Mg addition, a possible “dilution effect” due to the increase of biomass. These results suggest that Mg fertilizer may improve protein content in legume-based food as much as nitrogen fertilizer³³ or reduce protein in grass-based food while raising leaf P as might a phosphorus fertilizer,³⁴ albeit with different mechanisms. The different responses of the two species can be attributed to the different biochemical roles of their main symbiotic microorganism, that is, rhizobium and AMF, as it is generally known that rhizobium can fix nitrogen from the atmosphere while AMF mainly delivers various soil mineral elements, especially P.

Phosphorus. These functional differences in microorganisms may also explain differences in the total P content of the two species' responses to Mg treatments, with the marginally significant increase of P in maize leaves but not seen in soybean leaves in response to Mg addition.

Magnesium. In previous studies, as in the present study, Mg treatments significantly increased Mg concentration in leaf,⁶ which can be beneficial to animal nutrition in decreasing³⁵ the risk of grass tetany of herbivores³⁶ and in plant foods destined

for human consumption, which have shown decreased Mg concentration over time, especially with high-yield cultivars.³⁷

C:N:P Ratios. The C:N ratio in leaf of Mg-treated soybean was 50% lower than in nontreated soybean leaf; by contrast, soybean leaf N:P ratio more than doubled with Mg treatment. This is primarily due to the increase of symbiotic N fixation seen in Mg-treated soybean. The decrease of C:P and N:P ratios in maize leaf with Mg addition was associated with an increase of leaf P. According to theories behind stoichiometry studies, decreased C:N (seen in our soybean plants with Mg addition) or C:P (seen in our maize plants with Mg addition) ratios in plants may improve C flow to herbivores.¹⁰ Significant correlations between stoichiometry and mutualistic microbes in this study suggest that the differences in leaf C:N:P stoichiometry between maize and soybean come primarily from their differing mutualistic interactions between plant and microbe that are enhanced by Mg fertilizer.

Conclusions. Our results suggest that moderate Mg fertilization improved plant growth and altered C:N:P stoichiometry primarily via enhancement of mutualistic interactions between host plant and microbes as well as by promoting root growth. The potential mechanisms underlying this positive effect of Mg addition include an enhanced photosynthetic production flow from source leaves that promotes root growth and “feeds” mutualistic microbes and, possibly, a direct, positive Mg effect on AMF and rhizobium development that delivers more nutrients to the host plants. Such a change of stoichiometry brought about by Mg addition may favor nutrient and energy transfer in food webs. Our study has important implications for understanding the influence of Mg fertilizer and its underlying mechanisms on plant quality and contributes information for farmland management strategies that could improve food quality and yield.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.6b05764.

Methods of soluble carbohydrate analyses; results of rhizobium nodule to root biomass ratio in soybean with Mg addition (Figure S1); soluble carbohydrate in leaf and roots of maize and soybean under Mg addition (Figure S2) (PDF)

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Notes

The authors declare no competing financial interest.

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