

Article

Arbuscular Mycorrhization Enhances Nitrogen, Phosphorus and Potassium Accumulation in *Vicia faba* by Modulating Soil Nutrient Balance under Elevated CO₂

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Abstract: Effects of arbuscular mycorrhizal fungi (AMF), elevated carbon dioxide (eCO₂), and their interaction on nutrient accumulation of leguminous plants and soil fertility is unknown. Plant growth, concentrations of tissue nitrogen (N), phosphorus (P), and potassium (K) in 12-week-old nodulated faba bean (*Vicia faba*, inoculated with *Rhizobium leguminosarum* bv. NM353), and nutrient use efficiency were thus assessed under ambient CO₂ (410/460 ppm, daytime, 07:00 a.m.–19:00 p.m./nighttime, 19:00 p.m.–07:00 a.m.) and eCO₂ (550/610 ppm) for 12 weeks with or without AM fungus of *Funneliformis mosseae* inoculation. eCO₂ favored AMF root colonization and nodule biomass production. eCO₂ significantly decreased shoot N, P and K concentrations, but generally increased tissue N, P and K accumulation and their use efficiency with an increased biomass production. Meanwhile, eCO₂ enhanced C allocation into soil but showed no effects on soil available N, P, and K, while AM symbiosis increased accumulation of C, N, P, and K in both plant and soil though increased soil nutrient uptake under eCO₂. Moreover, plant acquisition of soil NO₃[−]-N and NH₄⁺-N respond differently to AMF and eCO₂ treatments. As a result, the interaction between AM symbiosis and eCO₂ did improve plant C accumulation and soil N, P, and K uptake, and an alternative fertilization for legume plantation should be therefore taken under upcoming atmosphere CO₂ rising. Future eCO₂ studies should employ multiple AMF species, with other beneficial fungal or bacterial species, to test their interactive effects on plant performance and soil nutrient availability in the field, under other global change events including warming and drought.

Keywords: AMF; biomass production; carbon dioxide enrichment; nutrient absorption; soil nitrogen content; soil organic carbon



Citation: Shi, S.; Luo, X.; Dong, X.; Qiu, Y.; Xu, C.; He, X. Arbuscular Mycorrhization Enhances Nitrogen, Phosphorus and Potassium Accumulation in *Vicia faba* by Modulating Soil Nutrient Balance under Elevated CO₂. *J. Fungi* **2021**, *7*, 361. <https://doi.org/10.3390/jof7050361>

Academic Editor: Raffaella Maria Balestrini

Received: 16 April 2021

Accepted: 2 May 2021

Published: 5 May 2021

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1. Introduction

The atmospheric carbon dioxide (ACO₂) is predicted to exceed 550 ppm by the end of this century from the 416 ppm in March 2021 (<https://www.co2.earth>, accessed on 1 May 2021). An elevated CO₂ (eCO₂) concentration has a direct effect on photosynthesis, and thus enhancements of carbon fixation and dry matter accumulation [1–4]. Shoot and root biomasses under eCO₂ are increased by 20–30% across a wide range of crop species, such as wheat [5], rice [6], soybean [7], and tomato [8]. The beneficial effect of eCO₂ on dry matter accumulation not only caused changes in C, nitrogen (N), phosphorous (P), and potassium (K) concentration, but also nutrient cycling from soil to plants [9]. When carbon fixation under eCO₂ exceeds its ability to produce new sinks in plants, their photosynthetic rate decreases to balance source and sink capacity [2]. Moreover, increased

biomass productivity of plants needs more supply of nutrients to match their increased carbon assimilation under eCO₂ [9–11]. Soil nutrient availability might decrease over one-to-seven-year eCO₂ exposure owing to an increased nutrient demand by eCO₂-stimulated growth [12,13]. Decreases in soil nutrient supply modulate the magnitude of the eCO₂ effect on plant biomass [14]. An ecosystem under eCO₂ is not sustainable if no concomitant increases in N, P and K supply with the eCO₂ stimulated growth can be maintained [15,16]. As a result, the availability of soil nutrients plays crucial roles in determining the response of plants to eCO₂.

Arbuscular mycorrhizal fungi (AMF), the most widespread symbionts in nature, form obligate mutualistic associations with ~72% of terrestrial plant species [17]. Being dependent on host plant C, AMF received ~10–20% of recently photosynthetic C from host plants for their own growth. Meanwhile, AMF takes up and transfers significant amounts of N and P to the host plant, which improves plant growth, nutrient absorption, and water-use efficiency [18]. Numerous studies have shown that eCO₂ exhibited a significant positive effect on C₃ plants because of stimulated photosynthetic C assimilation, which increases the photosynthates transfer to roots of mycorrhizal plants, supporting symbiotic association [19–21]. In turn, the formation of AM generally increased leaf, stem, and root biomass production of the host plant under eCO₂ [22–24].

Nutrient concentrations in C₃ plant tissues generally decreased under eCO₂ [15,25,26]. A meta-analysis of 7761 observations showed that average 689 ppm eCO₂ decreased plant N, P, and K concentrations by 7%–15%, and N declined more than P and K [15,27]. However, a lower plant N, P, or K under eCO₂ could be ameliorated by AM symbioses. For instance, compared to 50% decrease in non-AM plants, total plant P under 710 ppm eCO₂ was only decreased by 22% in 56-d-old AM *Robinia pseudoacacia* [28]. Positive AMF effects on lettuce's K concentration were greater under 700 ppm eCO₂ than under ACO₂ [29]. Chen et al. showed that ¹⁵N and total N uptake by *Plantago lanceolata*, not by *Festuca arundinacea*, were enhanced by AMF inoculation under 730 ppm eCO₂, suggesting that AMF effects on plant N uptake were species-specific under eCO₂ [30]. Faba bean (*Vicia faba* L.), a leguminous food crop, associates with N₂-fixing bacteria and alleviates N deficiency to some extent under eCO₂ via symbiotic N₂ fixation [2]. However, at present, the interactions effect of eCO₂ and AMF on plant nutrition accumulation and its soil fertility in legumes remain unknown, which limits our understanding of the nutrient-limiting response of legume growth to eCO₂.

A constant daytime and nighttime ACO₂ concentration or doubled-ACO₂ concentrations have been applied for almost all studies involving the response of plants to future atmospheric CO₂ concentrations. However, due to plant CO₂ consumption and stronger wind turbulence, etc., at daytime, but higher soil and plant CO₂ release, etc., at nighttime, the atmospheric CO₂ concentrations at canopy height are usually higher at nighttime than at daytime in agricultural fields of Australia, Japan, and USA [31], and also in our onsite observation (2017–2019, 417 ± 16 ppm at daytime and 463 ± 27 ppm at nighttime) on the campus of Southwest University, Chongqing, China. As a consequence, plants should differentially respond to such contrasting daytime or nighttime atmosphere CO₂ concentrations. Hence, supplying higher CO₂ at nighttime than at daytime would presumably provide closer simulations of future atmospheric conditions. With comparisons to plants responding to different daytime and nighttime CO₂ concentrations in environmentally controlled glass-made chambers, the objectives of the present study were to quantify (1) changes in C, N, P, and K accumulation in the plant–soil system of nodulated faba beans (inoculated with *Rhizobium leguminosarum* bv. NM353) under eCO₂ and (2) capacity of AMF for enhancing growth and nutrient accumulation in fabas bean by facilitating nutrient uptake from soil under eCO₂.

2. Materials and Methods

2.1. Experimental Setup

The experiment was conducted in six temperature and humidity-controlled growth chambers (1.5 × 1 × 2.5 m) made by tempered glass (90% light transmission) at the National Monitoring Base for Purple Soil Fertility and Fertilizer Efficiency (29°48' N, 106°24' E, 266.3 m above the sea level) on the campus of Southwest University, Chongqing, China. The CO₂ concentrations inside the growth chambers were also automatically maintained by a CO₂ auto-controlling facility (DSS-QZD, Qingdao Shengsen Institute of CNC Technology, Shandong, China). The detailed information about the automatically controlled environment facility was described in our previous study [4]. The experimental pots (21 × 17 cm) were filled with 3.4 kg soil (Eutric Regosol, FAO Soil Classification System). The soil was air-dried, sieved by passing through a 2 mm mesh, and sterilized at 121 °C for 120 min. The texture of the soil was sandy loam with a pH 6.8, 10.56 g soil organic carbon kg⁻¹, 0.66 g total N kg⁻¹, 0.61 g total P kg⁻¹, 97 mg available N kg⁻¹, 17 mg available P kg⁻¹, and 197 available K mg kg⁻¹.

2.2. Experimental Design and Treatments

The experiment was a split plot design with atmospheric CO₂ concentrations as the main factor and mycorrhizal inoculation as the subfactor. The experiment involved two AMF inoculations (*Funneliformis mosseae*, AMF, and no *F. mosseae* as control, non-AMF), and two CO₂ treatments (current ACO₂, 410/460 ppm, daytime, 07:00 a.m.–19:00 p.m./nighttime, 19:00 p.m.–07:00 a.m. and eCO₂, 550/610 ppm as increased by ~33.33% of ACO₂). Three growth chambers were used for ACO₂ treatment, and the other three for eCO₂ treatment. A total of six growth chambers were in a completed random arrangement.

The AMF inoculum (*Funneliformis mosseae*) was obtained from Bank of Glomales in China, located in the Beijing Academy of Agriculture and Forestry, Beijing. The inoculum had 50 spores per gram of dry soil in a mixture of soil, mycorrhizal mycelia, and root segments. Each AMF pot was supplied with 20 g *F. mosseae* inocula at 5 cm soil surface depth. Each non-AMF pot received an equal amount of autoclaved (121 °C, 0.1 Mpa, 120 min) inocula and 5 mL filtrate (0.45 µm syringe filter, Millipore Corporation, Billerica, MA, USA) from this AMF inoculum to minimize differences in microbial communities. Two AMF pots and two non-AMF pots were placed inside each growth chamber for a total of six replicated pots to each CO₂ concentration treatment. Seeds of Faba bean (*Vicia faba* cv. 89–147) from Chongqing Academy of Agricultural Sciences were sterilized with 10% H₂O₂ for 20 min, rinsed with sterile water, and then pre-germinated on sterilized moist filter paper at 25/20 °C (day/night) for 60 h. The germinated seeds were soaked with liquid inoculate of *Rhizobium leguminosarum* bv. NM353 for 30 min and sown in each pot (four seeds per pot), and then 5 mL *R. leguminosarum* bv. NM353 liquid inocula was also added to each pot. Except for the CO₂ concentration, the chambers had similar growth conditions such as fertilization, light, air temperature, and humidity. The similar temperature and humidity between inside and outside the growth chambers were also automatically maintained by the above-mentioned CO₂ auto-controlling facility [4]. The photosynthetic active radiation (PAR) was supplied by the natural light. To minimize differences in growth conditions, the position of growth pots in each chamber was rotated once a week and shifted to another replicate chamber once fortnightly. In addition, all the growth pots were watered once with Hoagland solution to a total of 100 mg N, 50 mg P, and 75 mg K per pot and were maintained at 70% water-holding capacity for the whole growth period.

2.3. Harvest and Sampling

Plant and soil samples were collected 12 weeks after sowing and were combined from two pots in each chamber as one composite sample. Thus, each treatment had three composite samples from six pots. Plant tissues were divided into shoots (leaves and stems) and roots. A portion of fresh roots was stored in 50% ethanol to determine root AM

colonization. The remaining fresh roots, shoots, and nodules were dried at 105 °C for 30 min and then at 75 °C for > 48 h until at a consistent dry weight. Soil samples were air-dried for > 48 h for the determination of chemical properties.

2.4. Mycorrhizal Colonization

Root AMF colonization rate was determined after clearing roots in 10% (*w/v*) KOH and staining with 0.05% (*v/v*) trypan blue in lactic acid [32]. Briefly, 50 stained segments were randomly selected and mounted on microscope slides to assess mycorrhizal root colonization. The root segments were checked under a microscope at 200 magnification. Mycorrhizal colonization rate was calculated by the percentage of infected root segments numbers out of the total number of observed root segments.

2.5. Determination of Carbon, Nitrogen, Phosphorus, and Potassium in Plants and Soils

The oven-dried shoot and root samples were ground into fine powder and then digested with 98% sulfuric acid and 30% hydrogen peroxide. Plant N concentrations were determined using the micro-Kjeldahl method, P concentrations using the vanadium molybdate yellow colorimetric method, and K concentrations by the flame photometry [33]. Carbon concentration was determined using the potassium dichromate-sulfuric acid oxidation method [33]. Soil available N was measured by the micro-diffusion technique after alkaline hydrolysis, and soil available P was extracted with 0.5 M NaHCO₃ and measured by the Mo-Sb anti spectrophotometric method [33]. Soil available K was determined by the flame photometry after extracting with 1.0 M ammonium acetate [33]. Soil NO₃[−]-N and NH₄⁺-N concentrations were analyzed using a continuous flow analyzer (Seal Auto Analyzer III, Hamburg, Germany) according to the company's manual after extracting with 1.0 M potassium chloride.

Plant tissue nutrient accumulation was calculated as tissue nutrient concentration multiplied with biomass production. The nutrient use efficiency (g) = (biomass production, g plant^{−1})² / (nutrient accumulation, mg plant^{−1}) was expressed as biomass produced squared per unit of a certain nutrient accumulated [34].

2.6. Statistical Analysis

Data were statistically analyzed using the SPSS Statistics 19.0 (StatSoft Inc., Tulsa, USA). Results were presented as means ± SE (*n* = 3). Significant differences among treatments were compared by the Tukey's Multiple Range Test at *P* < 0.05. Two-way analysis of variance (ANOVA) was conducted to determine the effects of CO₂, AMF colonization, and their interactions. The Pearson's correlation coefficients were calculated to assess the relationships between biomass productions and C, N, P, and K concentration in plant tissues and soils.

3. Results

3.1. Mycorrhizal Colonization, Nodule Biomass, and Plant Growth Parameters

Root colonization by *F. mosseae* was significantly increased by eCO₂, while it was not detected in the plants with autoclaved *F. mosseae* inocula. Both AMF and eCO₂ significantly increased nodule biomass, and the highest nodule biomass was observed in AMF plants grown under eCO₂ (Table 1).

eCO₂ significantly increased shoot, root, and total plant biomass production and decreased root/shoot ratio, regardless of whether the faba beans were colonized by *F. mosseae* or not (*P* < 0.01, Table 1). Compared to the non-AMF plants, *F. mosseae* colonization increased shoot and total plant biomass production under both ACO₂ and eCO₂ (*P* < 0.01, Table 1), but not for root biomass production and root/shoot ratio (*P* > 0.05, Table 1). The interaction between CO₂ and AMF was not significant, except for the nodule biomass production (*P* > 0.05, Table 1).

Table 1. Percentages of arbuscular mycorrhizal (AM) colonization, nodule biomass production, shoot biomass production, root biomass production, root/shoot ratio, and total biomass production in *Funneliformis mosseae* inoculated faba beans grown for 12 weeks at ambient CO₂ (ACO₂) and elevated CO₂ (eCO₂).

Treatment	AMF Inoculation	AM (%)	Nodule Biomass (g plant ⁻¹)	Shoot Biomass (g plant ⁻¹)	Root Biomass (g plant ⁻¹)	Root/Shoot Ratio	Total Biomass (g plant ⁻¹)
ACO ₂	M ⁻	0	0.04 ± 0.01b,x	1.91 ± 0.04b,y	1.01 ± 0.09b,x	0.53 ± 0.06a,x	2.91 ± 0.07b,y
	M ⁺	43.01 ± 2.49b	0.05 ± 0.01b,x	2.15 ± 0.08b,x	1.13 ± 0.08b,x	0.57 ± 0.05a,x	3.18 ± 0.09b,x
eCO ₂	M ⁻	0	0.07 ± 0.00a,y	3.24 ± 0.09a,y	1.26 ± 0.05a,x	0.39 ± 0.01b,x	4.50 ± 0.08a,y
	M ⁺	59.64 ± 3.04a	0.13 ± 0.02a,x	3.69 ± 0.09a,x	1.39 ± 0.14a,x	0.37 ± 0.01b,x	5.05 ± 0.12a,x
ANOVA							
CO ₂		**	***	***	**	**	***
AMF			*	**	ns	ns	**
CO ₂ × AMF			**	ns	ns	ns	ns

Data (means ± SE, n = 3) followed by different letters indicate significant differences between CO₂ treatments for the same AMF inoculation (a, b) and between AMF inoculations for the same CO₂ treatment (x, y) at P < 0.05 as revealed by Tukey’s test. ANOVA: ns not significant; *, **, and *** significant at P ≤ 0.05, P ≤ 0.01, and P ≤ 0.001, respectively. M⁺ or M⁻, with or without *F. mosseae* inoculation.

3.2. Effects of AMF and CO₂ on Plant Carbon, Nitrogen, Phosphorus and Potassium Concentrations

Shoot and root C concentrations were generally significantly higher under eCO₂ than under ACO₂ in both AMF and non-AMF plants (P < 0.01, Figure 1A,E), while they were unaffected by *F. mosseae* inoculation under the same CO₂ (P = 0.74–0.76, Figure 1A,E). Except for shoot N concentrations under eCO₂, shoot and root N concentrations were significantly greater in AMF than non-AMF plants under both ACO₂ and eCO₂ (P < 0.05–0.01, Figure 1B,F). Concentrations of shoot P or K in both non-AMF and AMF plants were significantly more decreased under eCO₂ than under ACO₂ (P < 0.05, Figure 1C,D), whereas root P or K concentrations were unaffected by either eCO₂ or *F. mosseae* inoculation (Figure 1G,H). A significant CO₂ × AMF interaction was observed in shoot C and N concentrations only (P < 0.05, Figure 1A,B), but not in shoot P and K concentrations or in root C, N, P, and K concentrations (Figure 1).

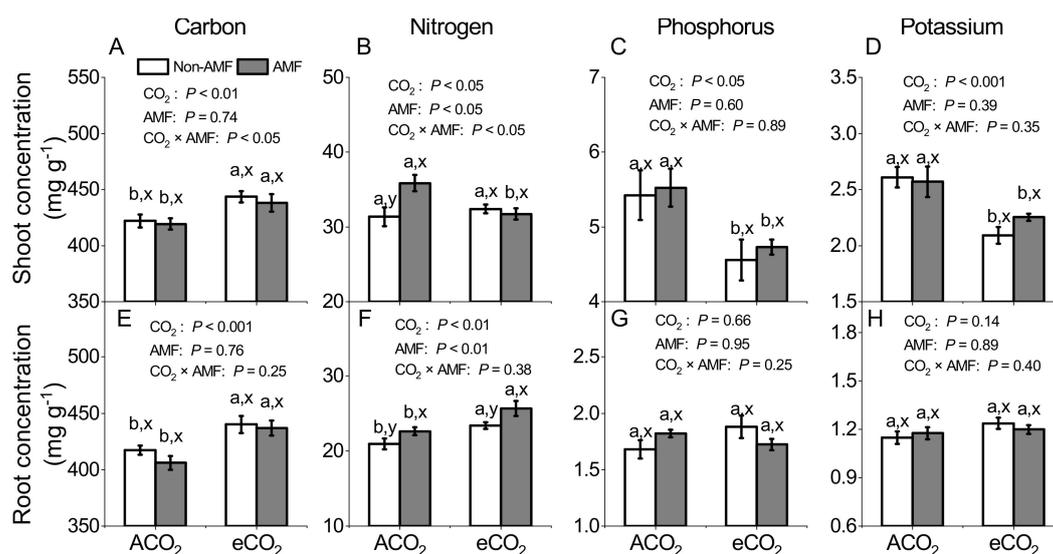


Figure 1. Concentrations of carbon (A,E), nitrogen (B,F), phosphorus (C,G), and potassium (D,H) in shoots and roots of *Funneliformis mosseae* inoculated faba beans grown for 12 weeks at ambient CO₂ (ACO₂) and elevated CO₂ (eCO₂). Data (means ± SE, n = 3) followed by different letters indicate significant differences between CO₂ treatments for the same AMF inoculation (a, b) and between AMF inoculations for the same CO₂ treatment (x, y) at P < 0.05 as revealed by Tukey’s test. Statistical comparisons (two-way ANOVA) between AMF or CO₂ treatments as well as their interaction (AMF × CO₂) are presented for each variable.

3.3. Effects of AMF and CO₂ on Plant Carbon, Nitrogen, Phosphorus, and Potassium Accumulations

Accumulations of C, N, P, and K in shoot, root, and total plant was increased more under eCO₂ than under ACO₂ in both AMF and non-AMF plants ($P < 0.05$, Figure 2). Compared with non-AMF plants, under eCO₂, not under ACO₂, AMF plants had greater shoot and total plant C, N, P, and K accumulations ($P < 0.05$, Figure 2A–D,I–L) and root N accumulations ($P < 0.05$, Figure 2F), but not root C, P, and K accumulations ($P = 0.18–0.66$, Figure 2E,G,H). In addition, no significant interactions of CO₂ × AMF were observed, no matter whether plants were grown under different eCO₂ or colonized by *F. mosseae* (Figure 2).

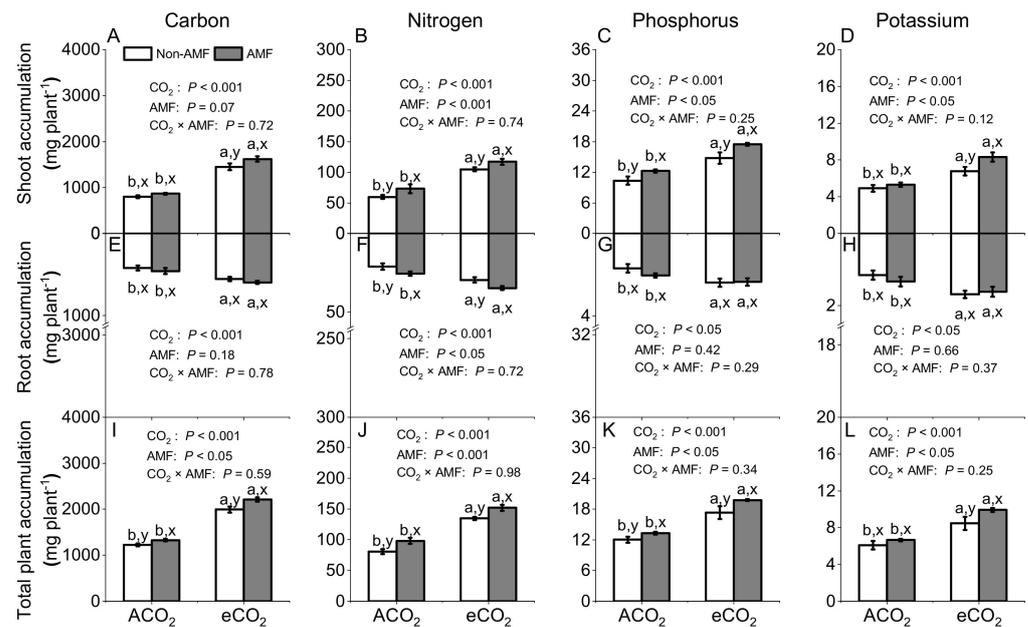


Figure 2. Accumulations of carbon (A,E,I), nitrogen (B,F,J), phosphorus (C,G,K), and potassium (D,H,L) in shoots, roots, and total plants of *Funneliformis mosseae* inoculated faba beans grown for 12 weeks at ambient CO₂ (ACO₂) and elevated CO₂ (eCO₂). Data (means ± SE, n = 3) followed by different letters indicate significant differences between CO₂ treatments for the same AMF inoculation (a, b) and between AMF inoculations for the same CO₂ treatment (x, y) at $P < 0.05$ as revealed by Tukey’s test. Statistical comparisons (two-way ANOVA) between AMF or CO₂ treatments as well as their interaction (AMF × CO₂) are presented for each variable.

3.4. Effects of AMF and CO₂ on Nutrient Use Efficiency

Compared to ACO₂, nutrient use efficiency of N, P, and K was significantly increased under eCO₂ in both non-AMF and AMF plants (Figure 3A–C). However, AMF colonization had no effect on N, P, and K use efficiency under the same CO₂ concentration. When combined with eCO₂ and AMF colonization, an increase in nutrient use efficiency of N, not P and K, was observed ($P < 0.05$, Figure 3A).

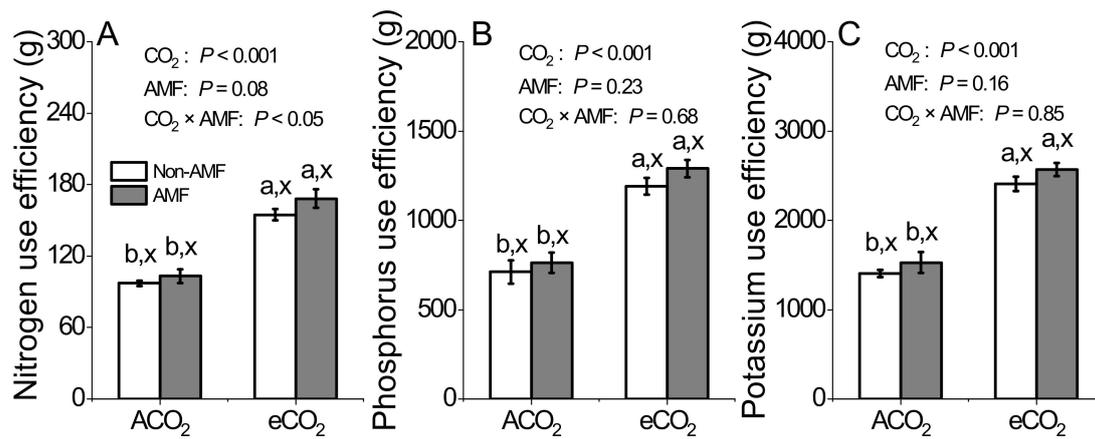


Figure 3. Nitrogen (A), phosphorus (B), and potassium (C) use efficiency in the total plants of *Funneliformis mosseae* inoculated faba beans grown for 12 weeks at ambient CO₂ (ACO₂) and elevated CO₂ (eCO₂). Data (means ± SE, n = 3) followed by different letters indicate significant differences between CO₂ treatments for the same AMF inoculation (a,b) and between AMF inoculations for the same CO₂ treatment (x, y) at P < 0.05 as revealed by Tukey’s test. Statistical comparisons (two-way ANOVA) between AMF or CO₂ treatments as well as their interaction (AMF CO₂) are presented for each variable.

3.5. Effects of AMF and CO₂ on Soil Nutrients

eCO₂ significantly increased soil organic carbon (P < 0.001, Figure 4A) and decreased soil NO₃⁻-N (P < 0.01, Figure 4D), but had no effects on soil available N (P = 0.06, Figure 4B), available P (P = 0.06, Figure 4E), and available K (P = 0.37, Figure 4F) irrespective of non-AMF or AMF colonization. Soil NH₄⁺-N was higher by 15.6% while lower by 19.7% under eCO₂ than ACO₂ in non-AMF and AMF soils, respectively (P < 0.05, Figure 4C). Meanwhile, AMF colonization significantly increased soil organic carbon, available N, NO₃⁻-N, available P, and available K under both eCO₂ and ACO₂ (P < 0.01, Figure 4). In addition, significant interaction of CO₂ × AMF was only in NH₄⁺-N (P < 0.001, Figure 4C).

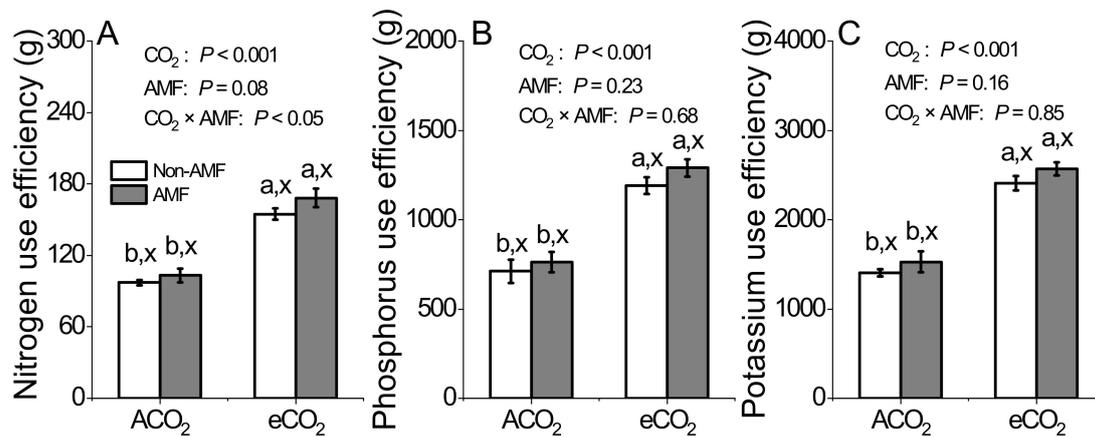


Figure 4. Soil organic carbon (A), soil available N (B), soil NH₄⁺-N (C), soil NO₃⁻-N (D), available phosphorus (E), and soil available potassium (F) in soils of *Funneliformis mosseae* inoculated faba beans grown for 12 weeks at ambient CO₂ (ACO₂) and elevated CO₂ (eCO₂). Data (means ± SE, n = 3) followed by different letters indicate significant differences between CO₂ treatments for the same AMF inoculation (a, b) and between AMF inoculations for the same CO₂ treatment (x, y) at P < 0.05, as revealed by Tukey’s test. Statistical comparisons (two-way ANOVA) between AMF or CO₂ treatments as well as their interaction (AMF × CO₂) are presented for each variable.

3.6. Correlations between Biomass Production and Plant Tissue or Soil Nitrogen, Phosphorus, and Potassium concentration

Shoot biomass production was significantly positively correlated to shoot C concentration (R² = 0.73, P < 0.05, Figure 5A), while negatively correlated to shoot N concentration

in non-AMF plants ($R^2 = 0.76$, $P < 0.05$, Figure 5D), but not in AMF plants ($P > 0.05$, Figure 5A,D). Shoot biomass production also significantly negatively correlated to shoot P or K concentration in both non-AMF and AMF plants ($R^2 = 0.55$ – 0.85 , $P < 0.05$, Figure 5G,J).

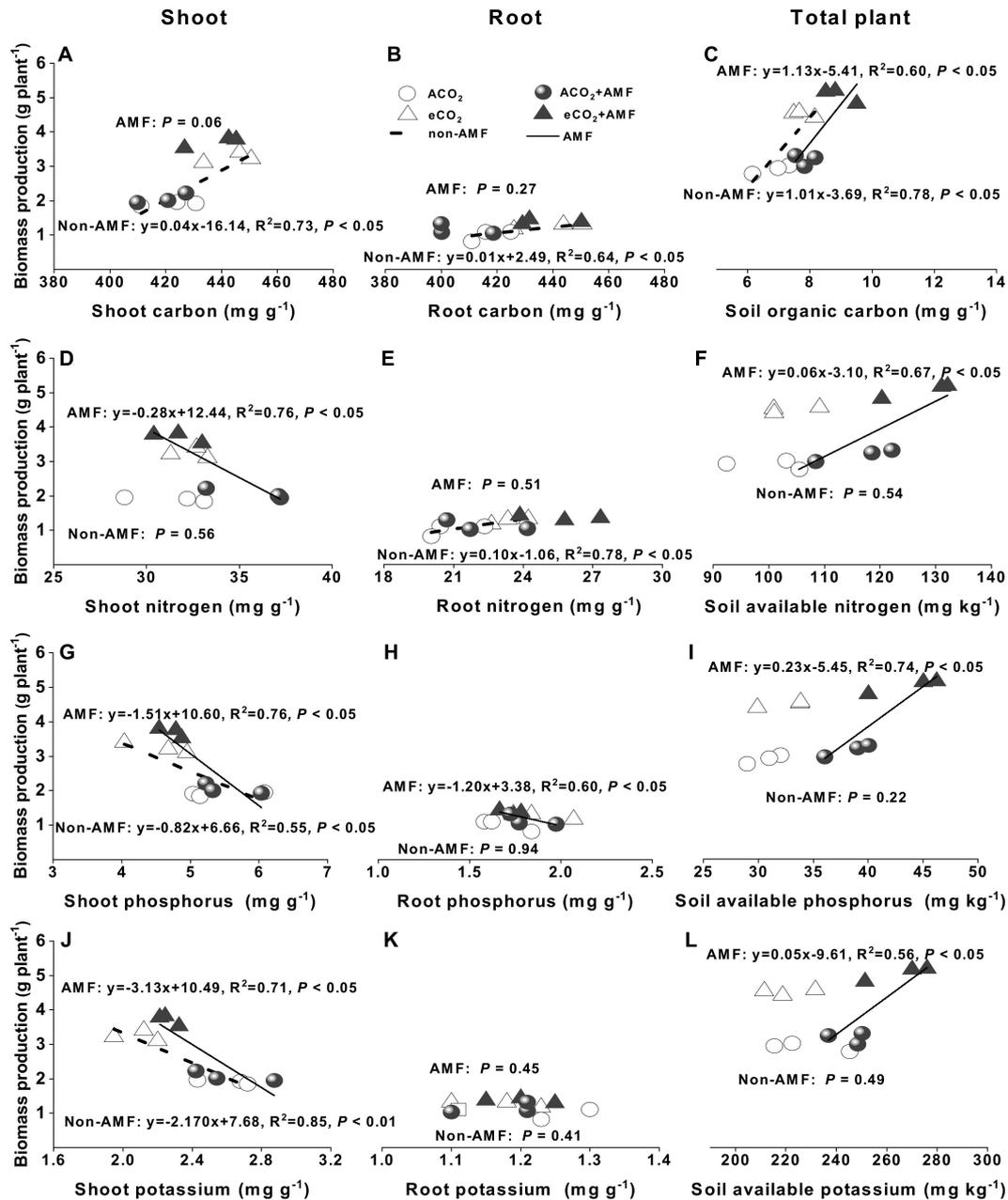


Figure 5. Relationships between carbon (A–C), nitrogen (D–F), phosphorus (G–I), and potassium (J–L) concentration in shoots, roots, and soils and plant biomass production of *Funneliformis mosseae* inoculated faba beans grown for 12 weeks at ambient CO₂ (ACO₂) and elevated CO₂ (eCO₂). Open circles and triangles represent data under ACO₂ and eCO₂ in non-AMF plants, and closed circles and triangles represent data under ACO₂ and eCO₂ in AMF plants, respectively. Regressions are shown for non-AMF (dotted lines) and for AMF (solid lines) treatments, $n = 6$.

Root biomass production was significantly positively correlated to root C or N concentration in non-AMF plants ($R^2 = 0.64$ – 0.78 , $P < 0.05$, Figure 5B,E), but not in AMF plants ($P = 0.27$ – 0.51 , Figure 5B,E), while significantly negatively correlated to root P concentration in AMF plants ($R^2 = 0.60$, $P < 0.05$, Figure 5H), but not in non-AMF plants ($P = 0.94$, Figure 5H). No relationships between root biomass production and root K concentration were observed in both AMF and non-AMF plants ($P = 0.41$ – 0.45 , Figure 5K).

Total plant biomass production significantly positively correlated to soil organic carbon in both AMF ($R^2 = 0.60$, $P < 0.05$) and non-AMF plants ($R^2 = 0.78$, $P < 0.05$, Figure 5C), and also to soil available N, available P, or available K in AMF plants ($R^2 = 0.56$ – 0.74 , $P < 0.05$), but not in non-AMF plants ($P = 0.22$ – 0.54 , Figure 5F,I,L).

4. Discussion

4.1. Mycorrhizal Colonization and Nodule Biomass Increased under eCO₂

The percentage of root colonization of faba beans by *F. mosseae* was significantly increased under eCO₂ (Table 1), in agreement with previous studies [20,24,35]. Compared to 336–400 ppm ACO₂, a meta-analysis from 434 observations demonstrated that 23% of extraradical hyphal length and 22% of mycorrhizal fungal biomass were increased under 550–1000 ppm eCO₂ [36]. The increased AMF colonization might be closely linked to an increased C allocation to their external hyphae and an enlarged root biomass production when plants were grown under eCO₂ [37–39]. Moreover, the nodule biomass under eCO₂ was significantly increased in both non-AMF and AMF plants (Table 1), and higher in AMF than in non-AMF plants (Table 1), suggesting that AMF did promote nodulation or N₂ fixation [40]. An improved photosynthesis under eCO₂ resulted in an increased translocation of photosynthates from shoots to roots [23,41,42], thus also favoring nodule development (Table 1). In turn, AMF and N₂-fixing bacteria received a certain amount of photosynthetic C from host plants to maintain their symbiotic interactions [2,18]. A similar nodule biomass in non-AMF and AMF plants under ACO₂ (Table 1) might be due to competitions for plant carbohydrate between AMF and N₂-fixing bacteria [40,41]. AMF was the dominant symbiont for photosynthetic C sink in the dual AMF/N₂-fixing bacterial symbiosis [40]. Once AMF colonization reached the plateau phase, more C was available for nodule growth [43] and consequently higher nodule biomass in AMF than in non-AMF plants under eCO₂.

4.2. AMF and eCO₂ Synergistically Improve Carbon Accumulation and Biomass Production

Studies have reported that AMF can promote plant growth under eCO₂ due to enhanced nutrient uptake and photosynthetic rate of the host plant [20,21,23]. Baslam et al. [42] found that both 700 ppm eCO₂ and AMF symbiosis increased shoot and root biomass production in vegetative nodulated alfalfa. Our results also showed that 550/610 ppm eCO₂ significantly improved shoot, root, and total plant biomass production (Table 1). Greater root biomass could be essential to sustain increased shoot biomass, since roots might be less efficient in transferring nutrients due to reduced transpiration-driven mass flow under eCO₂ [44]. For example, a greater effect of 700 ppm eCO₂ on faba bean roots at the stem elongation stage was observed in well-watered than in drought conditions [2]. However, the present results showed that eCO₂ generally stimulated more shoot biomass than root biomass production, leading to a significant decrease of root/shoot ratios in both AMF and non-AMF plants (Table 1). This finding agreed with results of Baslam et al. [21] in non-AMF alfalfa that 700 ppm eCO₂ increased the shoot to root ratios in both seven-week-old and nine-week-old plants, while all plants gradually increased their biomass partitioning to roots over the vegetative growth. Butterly et al. [26] showed 550 ppm eCO₂ significantly decreased the root–shoot ratio of field pea and wheat at grain filling but not at maturity [26]. These results suggested changes in biomass partitioning into roots under eCO₂ varied with plant growth stages. These alterations might be caused by the internal balance between labile N and C in the shoot and root [45]. A higher investment of photosynthates to roots could maintain better supply of nutrients through a well-developed root system for eCO₂ induced photosynthesis [46].

Shoot and root biomass production were significantly positively correlated to C concentrations in non-AMF plants ($R^2 = 0.64$ – 0.73 , $P < 0.05$, Figure 5A,B), indicating that an enhanced growth under eCO₂ was most likely due to an improved net photosynthetic rate [2]. AMF are known to receive photosynthetic C from host plants, and more C was available to roots under eCO₂ [24], leading to the enhanced photosynthesis rate as a

consequence of the increased C sink strength in mycorrhizal plants [40]. Such effects of mycorrhizal symbioses should reduce plant photosynthesis acclimation caused by carbohydrate accumulation when they were grown under long-term exposure to eCO₂ [47]. However, Gavito et al. [48] found no evidence of photosynthetic acclimation in *F. caledonium* pea grown under 700 ppm eCO₂ for nine weeks. In a greenhouse study, Goicoechea et al. [23] observed that *R. intraradices* association accelerated photosynthetic acclimation of alfalfa at the end of the vegetative stage under 700 ppm eCO₂. The C flow from host to AMF in roots might depend on the developmental stage of the different sinks within plants [49] or differences among AMF taxa in their exchange of carbon and nutrients [50]. Therefore, the effects of interactions between AMF and eCO₂ on photosynthetic acclimation, C accumulation, and dynamics merit further investigations.

4.3. AMF and eCO₂ Synergistically Improve Nitrogen Accumulation in Plant

It has been reported frequently that eCO₂ affects nutrient concentrations due to photosynthesis improvement, which was associated with the enhancement of the carboxylation reaction of RuBisCo [51]. Supporting such a concept, our results showed 11.8% of shoot N concentrations were decreased by eCO₂ in AMF plants, but not in non-AMF plants (Figure 1B). Bloom et al. [52] suggested that eCO₂ inhibited N assimilation and decreased N concentration in wheat and *Arabidopsis* plant. However this could not explain the reduction of shoot N concentration in AMF plants only (Figure 1B). The negative relationship between biomass production and shoot N concentration in AMF plants (Figure 5D) indicated that the reduced N might be ascribed to the increase in C assimilation and growth of AMF plants corresponding to plant N uptake, thereby reducing N concentration due to N dilution [24]. Moreover, we speculated that the mismatch between N demand and N assimilation by faba beans was overcome by a higher N uptake in a more developed root system under eCO₂. This was evidenced by a respective 12% and 16% increase of root N concentrations in non-AMF and AMF plants under eCO₂ (Figure 1F) and a significant relationship between root biomass production and root N concentrations ($R^2 = 0.78$, $P < 0.05$, Figure 5E). The increased root biomass and root N concentration under eCO₂ might lead to a higher proportion in root and a lower proportion of plant N in shoot. Drake et al. [53] suggested that the redistribution of N due to RuBisCo acclimation under eCO₂ could greatly increase N use efficiency. As the C assimilation was greater under eCO₂ than under ACO₂ (Figure 2A,E,I), the N, P, and K use efficiency was markedly increased in response to eCO₂ (Figure 3), while the increase in nutrient uptake rate might not keep up with biomass increase [34,54]. Nevertheless, nutrient use efficiency could be enhanced by each or all of the following three pathways: (i) biomass increase and constant nutrient absorption, (ii) constant biomass and lower absorption of nutrients, and (iii) increase in nutrient absorption rate being lower than that in dry mass increase rate [54].

Additionally, enhanced N accumulations in shoot, root, and total plant were observed under eCO₂ due to an increased biomass production (Figure 2B,F,J). Thus, plant N demand for C assimilation under eCO₂ was not reduced in the present study. AMF also significantly improved plant N accumulation regardless CO₂ level (Figure 2B,F,J). Similarly, a range of 30% to 41% increased N accumulation under 700–1000 ppm eCO₂ has been reported in AMF colonized alfalfa [23], *Plantago lanceolata* [30], *Taraxacum officinale* [20], and wheat [24]. Fellbaum et al. [55] reported that an increase of C supply to the host plant could stimulate N transport in AM symbiosis. In turn, AMF affected C translocation and dynamics in plant–soil systems [56]. Thus, AMF may regulate the demand for C and the supply for N to the host plant under eCO₂, and thus alter the balance of C and N.

4.4. AMF and eCO₂ Synergistically Improve Phosphorus and Potassium Accumulation in Plants

Concentrations of P and K under 550–750 ppm eCO₂ were increased in lettuce [29], *Oryza sativa*, and *Echinochloa crusgalli* [57], while they were reduced in *Artemisia annua* [58], tomatoes [8], and durum wheat [59], but not significantly changed in oregano [22] and mung bean [60]. Our results showed that shoot P and K concentrations were significantly

decreased under 550/610 ppm eCO₂ for both non-AMF and AMF faba beans (Figure 1C,D). Moreover, a significantly negative linear correlation between shoot dry biomass and P or K concentrations ($R^2 = 0.55\text{--}0.85$, $P < 0.05$, Figure 5G,J) was observed, suggesting that P or K concentrations were diluted by the increased shoot biomass production and such dilution effect also occurred in various C₃ plants under varied eCO₂ [15,19]. In addition, lower stomatal conductance and transpiration rates under eCO₂ might limit the transpiration-driven mass flow of nutrients through soil to root, thereby lowering the absorption of N, P, K, calcium, magnesium, and sulphur [45,61,62].

It is well known that AMF play important roles in the acquisition of essential nutrients including N, P, K, and other elements by through hyphal translocation [18]. Lower concentrations of N, P, magnesium, sulphur, zinc, and manganese in lettuce [29], tomato [8], and wheat [59] under 700–970 ppm eCO₂ have been shown to be mitigated by AMF inoculation. Compared to the non-mycorrhizal controls, significantly higher P and K accumulation in shoot and total plant displayed in the *F. mosseae* colonized faba bean regardless the CO₂ concentrations applied (Figure 2C,D,K,L), suggesting that AMF enhanced plant uptake of P and K. The higher biomass production and consequently greater nutrients demand of AMF plants under CO₂ might partly explain their increased nutrient accumulations. Similarly, Olesniewicz and Thomas [28] observed a significant increase of plant N and P in mycorrhizal *Robinia pseudoacacia* under 710 ppm eCO₂. In a study with two citrus spp., *Rhizophagus intraradices* colonization under 700 ppm eCO₂ stimulated plant growth and P acquisition of sour orange (*Citrus aurantium*), but not of sweet orange (*C. sinensis*) [63]. For *Pisum sativum*, however, the positive impact of AMF on shoot and total P disappeared under 700 ppm eCO₂ [48]. Therefore, effects of AMF inoculation on plant nutrient uptake could be species-specific at high CO₂ concentration.

4.5. eCO₂ has no Effect on Soil Nitrogen, Phosphorus, and Potassium Availability, but AMF Improves Soil Properties

Almost all eCO₂ studies have focused on plant characteristics and less on the feedback on the plant nutrient uptake and soil nutrients supply [12,13,56]. It is well-known that plant growth depends mainly on the nutrient reserves in soil [64], and eCO₂ might cause unpredictable repercussions on nutrient dynamics between soil nutrient pool and plant requirement [57]. When plants are grown under eCO₂, soil nutrient availability could decrease over longer time periods because of greater dry weights and more nutrient demands, resulting in a lower eCO₂ stimulation effect [65]. Our results showed that soil available N, available P, and available K concentrations were not significantly changed under eCO₂ (Figure 4B,E,F), suggesting that soil nutrients were not a limitation. The lack of response of soil nitrogen and phosphorus to eCO₂ (Figure 4B,E) and significantly increased nodule biomass under eCO₂ (Table 1) implied that soil P availability did not limit N fixation in the present study and the increased N₂ fixation could meet the part of plant N demand. However, it is still unknown that how much N could be fixed by N₂-fixing bacteria under eCO₂ and/or AMF. The determination of N₂-fixing could thus provide the understanding of the dynamics behind the positive impacts of AMF and eCO₂ on leguminous plants.

Furthermore, the present results showed that significantly higher soil organic carbon, available N, available P, and available K (Figure 4) were in AMF than in non-AMF soils, especially under eCO₂. This higher increase in soil organic carbon might be attributed to higher biomass production under eCO₂ [59]. eCO₂ allowed greater C investment to AMF and stimulated their biomass and activity [36,56]. In contrast, AMF increased the CO₂ fixation and induced the transport of carbon from plant to soil, thereby increasing soil organic carbon content [66]. A generally increase trend in soil organic carbon with AMF colonization was also synchronized in rice grown under 550 and 700 ppm eCO₂ [57]. Moreover, an increased AMF response to eCO₂ could result in increasing decomposition of complex organic material [66], and the increasing organic matter decomposition might hence accelerate soil nutrient mineralization rate, resulting in a better N and P availability to plants [67–69]. Therefore, it may not be necessary to apply more N, P, and K fertilizers to soil for faba beans' growth at their vegetative growth stage under eCO₂.

Although soil available N concentration was unresponsive to eCO₂, soil NO₃⁻-N and NH₄⁺-N were affected by either eCO₂ or AMF (Figure 4C,D). AMF can obtain both NH₄⁺-N and NO₃⁻-N and then transfer them to host plants [70]. Uptake of N in the form of NO₃⁻-N by AMF may play a major role in agricultural soils, where NO₃⁻-N is often the dominant N form [52]. In line with this, soil NO₃⁻-N was significantly higher in AMF than in non-AMF soils under both aCO₂ and eCO₂ (Figure 4D). In contrast, eCO₂ decreased soil NO₃⁻-N in both AMF and non-AMF soils (Figure 4D), but increased NH₄⁺-N in non-AMF soil only (Figure 4C). The decrease in soil NO₃⁻-N under eCO₂ was consistent with the result in poplar, in which 550 ppm eCO₂ negatively influenced nitrification by 44% under unfertilized treatment and consequently decreased NO₃⁻-N availability by 30% on average [71]. Meanwhile, ~560 ppm eCO₂ reduced the population of ammonia-oxidizing bacteria in soil and nitrification activities, leading to an increase of soil NH₄⁺-N and a decrease of the NO₃⁻-N [72]. Therefore, the CO₂-stimulated increase on biomass may be attributed to an increased uptake of NO₃⁻-N by the faba bean, thus depleting soil NO₃⁻-N pool, whereas the lower NH₄⁺-N observed under eCO₂ in AMF soils (Figure 4C) showed that a high plant demand for NH₄⁺ might be the primary driver for CO₂ enhancement of AMF-mediated organic matter decomposition [66].

5. Conclusions

Although eCO₂ generally resulted in reduction of shoot N, P, and K concentration, the nutrient use efficiency and accumulation of N, P, and K in shoot, root, and total plant were generally significantly increased. Meanwhile, eCO₂ enhanced C allocation into soil but did not reduce soil available N, P, and K. Compared to non-AMF treatment, AM symbiosis increased their accumulation of C, N, P, and K in both plant and soil though increasing organic matter decomposition and soil nutrient uptake under eCO₂. Moreover, soil NO₃⁻-N under eCO₂ was significantly higher, but soil NH₄⁺-N was lower in AMF than in non-AMF soils, suggesting that plant acquisition of soil NO₃⁻-N and NH₄⁺-N responded differently to AMF and eCO₂ treatments. As a result, the interaction between AM symbiosis and eCO₂ did improve plant C accumulation and soil N, P, and K uptake in faba bean, and an alternative fertilization programs for legume plantation should be hence taken under upcoming atmosphere CO₂ rising scenarios. Further eCO₂ studies should employ two or more mycorrhizal fungal species, either individual or multi-species combined, also with other fungal or bacterial species, to test their interactive effects on both plant growth and yield production, and soil nutrient availability in the field, under other global environment change scenarios, such as warming or temperature rising, drought, and so on.

Author Contributions: S.S. and X.H. conceived and designed the experiments; X.L., X.D., C.X., and Y.Q. performed the pots experiments and collected the samples. X.L. and X.D. were responsible for determination of root AMF colonization and C, N, P, and K concentration in plants. C.X. and Y.Q. analyzed the soil properties. X.H. and S.S. provided fund support and wrote the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Natural Science Foundation of China (4111800096), Science and Technology Department of Sichuan Province, (2018JZ0027), Biological Science Research Center at Southwest University (100030/2120054019) and Chongqing Key Laboratory of Plant Resource Conservation and Germplasm Innovation, China.

Informed Consent Statement: Not applicable.

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

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