

Plant Availability of Magnesium in Typical Tea Plantation Soils

Qunfeng Zhang (✉ still_hill@126.com)

Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310058. Key Laboratory for Plant Biology and Resource Application of Tea, the Ministry of Agriculture, China.

<https://orcid.org/0000-0002-2301-1816>

Dandan Tang

CAAS: Chinese Academy of Agricultural Sciences

Xiangde Yang

CAAS: Chinese Academy of Agricultural Sciences

Saipan Geng

CAAS: Chinese Academy of Agricultural Sciences

Ying He

CAAS: Chinese Academy of Agricultural Sciences

Yupei Chen

CAAS: Chinese Academy of Agricultural Sciences

Xiaoyun Yi

CAAS: Chinese Academy of Agricultural Sciences

Kang Ni

CAAS: Chinese Academy of Agricultural Sciences

Meiya Liu

CAAS: Chinese Academy of Agricultural Sciences

Jianyun Ruan

CAAS

Research article

Keywords: Mg fertilization, tea plant, plant-available, Mg efficiency, plant availability of Mg

Posted Date: December 4th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-117886/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background and aims: Magnesium (Mg) plays important roles in improving the yield and quality of tea. However, Mg deficiency frequently occurs in acidic tea plantation soil.

Methods: Tea plants were pot-cultivated in 12 typical tea plantations soils amended with and without Mg fertilizer. Exchangeable Mg (Ex-Mg) concentration in soils were quantitatively extracted using four extraction solutions (Mehlich-3, BaCl₂, CaCl₂ and NH₄OAC). Plant availability of Mg was evaluated by Mg uptake and use efficiency, as well as its association with quality-components in tea plants.

Results: Ex-Mg in soils were extracted most efficiently by Mehlich-3, while Mg concentrations in tea plant tissue higher correlated with Ex-Mg extracted by CaCl₂ than other extraction solutions. Mg fertilizer use efficiency in tea plant varied from 6.08% to 29.56 %, and Mg fertilization significantly improve green tea quality by decreasing the ratio of total polyphenol to amino acid in tea leaves (24-60%). Moreover, the effect of Mg application on tea quality improvement and the use efficiency of Mg fertilizer both negatively correlated with total Mg concentration ($r = -0.94$ and -0.63 , respectively) and nitrogen (N) level ($r = -0.61$ and -0.51 , respectively) in soils prior to tea plant cultivation.

Conclusions: CaCl₂ could be recommended for plant-available Mg extraction in tea plantation soil, and Mg fertilizer use efficiency could be affected and predicted by total N and Mg status in soils prior to tea plant cultivation, providing a potential theoretical for guidance of Mg fertilization for tea yield and quality improvement in tea plantation management.

Introduction

Magnesium (Mg) as the second most abundant intracellular cation has been proven to have vital functions in plants because Mg participates in chlorophyll synthesis and catalytic action (Gerendás and Führs, 2013). In recent years, considerable progress has been made in studying the effect of Mg fertilization on the yield and quality of cash crops (Cakmak et al. 2013; Dechen et al. 2015). In tea plant, previous study has shown that the use of Mg fertilizer can effectively improve the yield and quality of tea (Ruan et al. 1999). Our early experiment found that Mg application significantly increased caffeine content and aroma components in tea product (Ruan et al. 1999). Besides, Ruan et al. (2012) also suggested that improving Mg nutrition in tea planting effectively decrease the ratio of total polyphenols to amino acid (TP/AA), which is positively and strongly connected to green tea taste (Zhang et al. 2016). However, there is a deep gap in the knowledge of crops' requirements for Mg (Grzebisz et al. 2010).

Soil Mg incorporated in the crystal lattice structure of minerals cannot be directly absorbed by plant (Senbayram et al. 2016). In general, plants prone to absorb the readily Mg from the soil solution. Exchangeable Mg is an important source of plant available Mg. In the agricultural production system, the availability of Mg to crops depended on various factors such as soil texture, cation exchangeable capacity (Hariadi and Shabala, 2004), site specific climatic and anthropogenic factors, agronomic

management practices, as well as crop species itself (Mikkelsen, 2010; Scheffer and Schachtschabel, 2002).

Adequate soil Mg is a key to ensure robust crop growth and production, and soils with low concentrations of Mg can decrease agricultural productivity and quality (Wang et al. 2020). Mg losses by mobilization and leaching in the soil (Schachtschabel, 1954), or Mg depletion due to intensive crop production (Pol and Traore, 1993). Additionally, cationic competition, resulting from long-term imbalanced soil fertilization, causes nutrient heterogeneity in soils. A good soil Mg condition is the prerequisite to ensure Mg uptake by crop roots and enhance Mg utilization efficiency. Mg deficiencies in soil are a major limiting factor for crop growth (Cakmak and Yazici, 2010; Cakmak, 2013; Guo et al. 2016), particularly in acidic soils that are highly saturated with cations, such as H^+ , Al^{3+} and Mn^{2+} , and which suffer intensive leaching, particularly in areas with high rainfall totals (Gransee and Führs, 2013; Mayland and Wilkinson, 1989; Wilkinson et al. 1990). Tea plantations are generally located in regions with acidic soil, and Mg deficiency is an important limiting factor for tea production, both in terms of yield and quality (Wu and Ruan, 1994). At present, however, the input of Mg fertilizer to tea plantations is insufficient (Ni et al. 2019; Ruan et al. 2002).

The availability of Mg also depends on the activity or proportion of Mg relative to soluble and exchangeable amounts of K, Ca, Na, Al and Mn. Previous studies have reported that the status of nutrients such as potassium (K) (Farhat et al. 2013) or Nitrogen (N) (Ruan and Gerendas, 2015) is closely related to the biological effects of Mg application (Lasa et al. 2000). The cooperative of Mg and N nutrition on crops has been widely reported (Ruan et al. 2000; Ruan et al. 2004). For example, a hydroponics experiment with tea plants showed that the effects of Mg on tea quality were limited by N nutrition (Ruan et al. 2012). Moreover, Mg supply could improve the absorption and metabolism of nitrate in tea plants (Ruan et al. 1998) and promotes the long-distance transportation of amino acids and sugars in the xylem and phloem of tea plants (Ruan et al. 2012). Excessive application of high rates of K^+ and NH_4^+ fertilizers, antagonistically interferes with plant Mg uptake, thus enhancing the risk of Mg deficiency.

Recent years, studies of the molecular mechanisms of Mg metabolism (Deng et al. 2006) and the need for Mg in the field have produced much new knowledge (Conn et al. 2011; Senbayram et al. 2016). In particular, the understanding of the plant availability of Mg for plant growth has deepened (Gransee and Führs, 2013). However, the challenge is to relate the data obtained from soil analysis to the phytoavailability and plant growth (Kopittke and Menzies, 2007). Nutrient balance is considered as a simple diagnostic procedure, evaluating a current status of crop nutrient management. Two main approaches, the soil surface balance (SSuB) and the soil system balance (SSyB), are used to assess trends in a nutrient balance during fixed period. SSuB relies on a net balance of an external input and output of a given nutrient While SSyB relies on both external and internal (soil) resources (Oenema et al. 2003). In addition, the efficiency of a given extraction is shown by the correlation between the extractable Mg content and crop uptake. A good extraction should simulate the capacity of the roots to uptake nutrients, to extract as many elements of interest as possible, and to be suitable for the prediction of extractable Mg in chemically and physically different soils (Van Raij, 1988). The Mehlich 1 acid solution

(Nelson, Mehlich, and Winters 1953), Mehlich 3 solution (Mehlich, 1984) and ion-exchange resin (IER) (Van et al.1986) are commonly used as extractants. In some cases, the Mehlich 3 solution has shown better correlation between extracted P and plant uptake, when compared to Mehlich 1 (Tran et al. 1990).

In this study, to evaluate plant availability of Mg under different soil nutrients status, we collected soils from 12 tea-producing areas of China to cultivate tea plants in greenhouse supplied with and without additional Mg nutrient. Our main hypothesis is that the plant-available Mg in tea plantation soil depends on the Mg status (chemical-available, ect.) and soil characteristics (Nutritional status, ect.).

Materials And Methods

Experimental design

Surface soils (0-20 cm) were collected from 12 typical tea plantations located in main tea-producing areas in China, the detailed information of collected soils was shown in Table 1. Prior to the experiment, soil samples were air-dried, ground, sieved, and mixed with quartz (1:1, v : v). Then four 1-year-old tea clones (Longjing-43) were selected to cultivate in the mixed soils with a 350 mL plastic cup (bottom drainage). All pots were evenly placed in the climate chamber (Figure S1). The air temperature and relative humidity were maintained at 26/22 °C in the photo/dark-period and 70%, respectively. The photo/dark period was 14/10 h and the light intensity was 200 mmol m⁻² s⁻¹. During experiment, 50 mL deionized water was added to plots every 3 days and soil leachate was also periodically collected with plastic cup for recycling use. After 2 months of pre-incubation, tea plants were divided equally into two groups. Experimental groups (+Mg group) supply Mg equivalent to 40 mg MgSO₄. After 6 months of cultivation, the root, stem and leaves were sampled, respectively. Plant samples were frozen immediately with liquid nitrogen and freeze-dried, respectively. Root and stem were oven-dried at 60 °C and then ground using a ball mill (M301, Retsch, Germany). Additionally, the remaining soils were then air-dried (Figure S2) and passed through a 2-mm mesh sieve to remove the quartz from soil.

Analysis of soil samples

Soil pH, particle size distributions, total carbon (C) and N contents

Soil pH was measured at a ratio of 1:1 (w/v) in deionized water with an ORION 3 STAR pH meter (Thermo Fisher Scientific, Waltham, MA, USA). The particle size distributions were analyzed using a laser particle size analyzer (Mastersizer 3000, Malvern, UK). Total C and N contents were measured with an elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Each soil had three replicates and three technical replicates were calculated for mean values.

Total Mg and exchangeable Mg (Ex-Mg)

To measure total Mg content in soils, 0.2 g soil (pass through 100 mesh sieve) was digested with 5 mL nitric acid, 1 ml perchloric acid and 2 ml hydrofluoric acid at 140°C for 3h. Mg concentration in the solution after dilution was analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES, Thermo Jarrel Ash, USA).

The Ex-Mg content was extracted with 0.01 M BaCl₂, Mehlich-3 solution, 1 M CaCl₂ and 1 M ammonium acetate, respectively, at a ratio of 1:10 (w/v) for shaking 30 min at 180 r min⁻¹, and passed through 0.45-µm-sized cellulose–acetate paper filters for the measurement of Mg concentration by ICP-AES (Yang et al. 2018).

Analysis of tea plant samples

Mg concentrations in tea plant

The samples of tea plant (0.1g) were digested by 5 mL mixed concentrated acids HNO₃–HClO₄ (Ruan et al. 2012) at 140°C in an electric oven. The digestion was then diluted to 25 mL with distilled water and the nutrients were measured by ICP-AES.

Total polyphenols, free amino acids and chlorophylls in tea leaf

To measure total polyphenols, 60 mg finely ground tea plant powder was extracted with 3 mL deionized H₂O in a boiling water bath for 5 min (with vortex mixing for 2.5 min). A Folin–Ciocalteu colorimetric assay, with gallic acid as the reference standard, was used to spectrophotometrically determine its concentration in the filtrate (ISO14502-1).

The free amino acids in tea leaves were measured via high-performance liquid chromatography with a diode array detector (HPLC-DAD, Waters, 2695–2998), as previously reported by Zhang et al. (2018).

Chlorophyll content determination: 100 mg powdered leaves was extracted with 5 mL acetone and ethanol (1:1, v:v) and protected from light for 24 h. The extraction was spectrophotometrically determined after centrifugation and filtration. The chlorophyll a and b contents were derived using the Arnon formula.

Data analysis

Statistically significant differences in mean values were tested using one-way ANOVA. Tukey post hoc test was applied for comparison of multiple groups. Pearson correlation and linear regression analysis were test with SigmaPlot 12.0 (Systat Software, USA). Mg fertilizer use efficiency was concluded with the formula as:

$$UE = \frac{(C1 * M1 - C2 * M2) + (C3 * M3 - C4 * M4) + (C5 * M5 - C6 * M6)}{TM} * 100\%$$

Where, UE means Mg fertilizer use efficiency. C1, C3 and C5 means Mg concentrations of tea root, stem, leaf respectively in +Mg group plants. M1, M3 and M5 means biomass of tea root, stem, leaf respectively in +Mg group plants. C2, C4 and C6 means Mg concentration of tea root, stem, leaf respectively in CK group plants. M2, M4 and M6 means biomass of tea root, stem, leaf respectively in CK group plants. TM means total amount of Mg fertilizer applicate in each pot.

Results

Soil Ex-Mg concentration prior to experiment

Ex-Mg concentration in soils extracted by different extraction solutions vary widely. For example, Ex-Mg concentration extracted by M₃ solution in XY site was three times more than CaCl₂-extracted concentration of Ex-Mg (245.04 and 72.85 mg kg⁻¹ respectively). However, the concentration of soil Ex-Mg in XY, Xsbn, WX, CD, HG, YC, QY and CS sits were highly extracted by M₃ solution, and followed by decreasing order of BaCl₂, NH₄OAC and CaCl₂. While CaCl₂ solution showed higher soil Ex-Mg extraction efficiency in XX, ND and CQ sites (Figure 1a). Herein, simple linear regression was applied to model the relationship between the methods of M₃, BaCl₂, CaCl₂ and NH₄OAC. The results showed that M₃ solution had the strongest correlation with NH₄OAC (r² = 0.86, p < 0.001), followed by BaCl₂ solution (r² = 0.83, p < 0.001), by contrast, soil Ex-Mg concentration extracted by CaCl₂ solution showed a lower correlation with NH₄OAC (r² = 0.68, p < 0.001) (Figure 1b).

Mg concentration in tea plants

Among three tissues, the highest Mg concentration occurred in leaf, followed by root and stem (Figure 2). Mg application significantly increased Mg concentration in the roots by 20%, stems (83%) and leaves (36%), respectively (Figure 2a, b, c). Besides, the effect of Mg application on tea plant varied dramatically due to the initial status of soil Mg nutrition. For example, tea plants cultivated in soils originating from XY site had the highest Mg concentration (3.5, 1.9, 4.3 mg g⁻¹ in the roots, stems and leaves, respectively, Figure 2), which is consistent with Ex-Mg changes in soils (245.6±1.6 mg kg⁻¹), by contrast, lower level of Mg were measured in tea plants tissue (0.92, 0.90, 2.02 mg g⁻¹ in the roots, stems and leaves, respectively, Figure 2) which cultivated in soils of CQ with less Ex-Mg (30.7±1.5 mg kg⁻¹) than XY. Pearson correlation results (Table 2) showed that the Mg concentration in root, stem and leaf significantly and positively correlated with initial soil Ex-Mg concentration (prior to tea plant cultivation). Compared with root tissue (r < 0.6), the Mg concentration in stem and leaf highly correlated with soil Ex-Mg (r > 0.6). Likewise, chl_a and

chl_b also significantly and positively correlated with Ex-Mg. Compared with chl_a, the relationship between chl_b and Ex-Mg was relatively weak, especially Ex-Mg extracted by CaCl₂ solution ($r = 0.178, p > 0.05$).

After tea plant cultivation, the nutrient Mg, especially Ex-Mg, could be absorbed directly by the root, and then transport to other tissue like stem and leaf. Indeed, there is a significant correlation ($r = 0.447 - 0.906, p < 0.01$) between Mg concentration among tissue (root, stem and leaf) and soil Ex-Mg concentration, whether it is in CK group or Mg group (Table 2). Both CK group and Mg group, there was a larger correlation between the Mg concentration in tissue and soil Ex-Mg ($r = 0.499 - 0.906$). Moreover, soil Ex-Mg concentration extracted by CaCl₂ had the strongest correlation with Mg concentration in plant tissue (root: $r = 0.636$ and 0.650 ; stem: $r = 0.733$ and $r = 0.640$; $r = 0.906$ and $r = 0.740$, in CK and Mg group, respectively). Different from Mg concentration in plant tissue, a weak correlation was found between chlorophyll (chl_a, chl_b) and soil Ex-Mg concentration, but only chl_b positively correlated with soil Ex-Mg extracted by BaCl₂ ($r = 0.359, p < 0.05$).

The changes in soil Ex-Mg pre- and post- tea plant cultivation was defined as Δ Mg. The results showed that the Mg concentration in tissue negatively correlated with Δ Mg-M₃ ($r = -0.428, r = -0.475$ and $r = -0.413$ in root, stem and leaf, respectively), but positively correlated with Δ Mg-CaCl₂ ($r = 0.628, r = 0.702$ and $r = 0.917$ in root, stem and leaf, respectively). However, there is no significant correlation between Mg concentration in plant tissue and soil Ex-Mg-NH₄OAC and Ex-Mg-BaCl₂. Both the content of chl_a and chl_b had significant negative correlation with soil Ex-Mg which was extracted by M₃ and NH₄OAC solutions. Whereas, they have no significant correlation with soil Ex-Mg which was extracted by CaCl₂ and BaCl₂ solution.

The determination of tea quality (amino acids (AA), total polyphenols (TP) and TP/AA)

Compared with the control treatment, Mg input significantly increased the total contents of AA (by 7-88%, mean was 55%) but decreased those of TP (by 2-32%, mean was 15%). Therefore, the ratios of TP/AA in all samples were significantly decreased (by 24-60%, mean was 44%) with Mg fertilization (Figure 3).

Linear regression analysis showed that the ratio of TP/AA significantly and positively correlated ($r = 0.66, p < 0.01$, Figure 4a) with soil initial total Mg contents value under Mg application. Whereas, in the CK group, the ratio of TP/AA showed an insignificant correlation ($r = -0.001, p = 0.99$, Figure 4a) with soil initial total Mg content. The correlation between TP/AA ratio and soil Ex-Mg were negligible ($r = 0.09, 0.005; p = 0.06, 0.67$ in CK and Mg group respectively, Figure 4b). On the other hand, without Mg application, the ratio of TP/AA in tea leaf negatively correlated with soil initial total N without Mg application ($r = -0.73, p < 0.001$, Figure 4c). However, there is no significant correlation between TP/AA ratio and N contents in soil ($r = 0.003, p = 0.75$, Figure 4c) with Mg application, indicating Mg fertilizer input in N- deficiency soils could have higher efficiency than in N- rich soils (Figure 4e).

Use efficiency of Mg fertilizer in tea plantation soil

The proportion of Mg taken up in plant tissue from the Mg supply were 1.30 %, 10.17 %, and 3.84 %, in root, stem and leaf respectively (Table 3). Among 12 different regions soils, the highest and lowest of Mg fertilizer use efficiency were WX (29.56) and YC (6.08) sites, respectively. Based on Linear regression, the Mg fertilizer use efficiency in tea plant negatively correlated with soil initial total Mg content (Figure 5a, $r = -0.63$, $p < 0.05$), similar, it negatively correlated with soil initial total N content (Figure 5b, $r = -0.51$, $p < 0.05$). However, there was no significant correlation between Mg fertilizer use efficiency and Ex-Mg in soils ($r = -0.035$, $p = 0.91$).

Discussion

Plant-available and chemical-available Mg in tea plantation soil

It is generally accepted that soil chemical analysis is a common and rapid method for evaluating soil nutrient status. However, the efficiency of different extractions varied greatly due to their strengths of ion exchange (Iatrou et al. 2015; Zbiral, 2016). Based on comparing six different Mg extraction methods, Staugaitis and Rutkauskienė (2010) found that mild extraction procedures including CaCl_2 , KCl, NH_4OAc and Mehlich 3 method showed similar extraction effect. Nelson et al. (1972) reported that the ammonium acetate (NH_4Ac) method extracted more Mg from soils with ranging neutral to alkaline conditions, high clay, and high organic matter content than by double acid method, while the latter gives erroneously high results if soils contain dolomitic limestone or other acid-soluble Mg. In this study, four extraction methods including CaCl_2 , KCl, NH_4OAc and Mehlich 3 showed high correlations, and Ex-Mg was extracted most efficiently by Mehlich 3. Indicating the importance of choosing an appropriate extraction method for correct evaluation of the Mg availability to crops.

Soil chemical analysis only indicate the potential nutrient supply capacity of soil for plant, plant availability could be greatly affected by many biotic and abiotic factors, such as nutrient mobility, root growth, and rhizospheric microorganisms (Dragicevic et al. 2016; Soremi et al. 2017; Yousaf et al. 2016). Which means a good extraction procedure should exhibit a high correlation with the total nutrient (Mg) uptake, while the absolute amount being extracted is irrelevant. However, previous study suggested that there is often a poor relationship between the plant growth response and extractable nutrients in the soil (Gransee and Führs 2013; Ortas et al. 1999;). In this study the concentrations of Mg in tea plant tissue were largely correlated with Ex-Mg extracted by CaCl_2 than other chemical extractions (BaCl_2 , NH_4OAc and Mehlich 3). Indicating that Ex-Mg extracted by CaCl_2 strongly correlated with the plant availability of Mg in tea plantation soil. In agreement with Papenfuß and Schlichting (1979) results that soil Ex-Mg extracted by CaCl_2 can substantially contribute to plant Mg nutrition. Early result suggested that water-soluble and Ex-Mg fractions in soils could not always reflect the crops' capacity to mine the soil (Gransee and Führs, 2013), therefore, evaluation of the contribution of different soil Mg pools to plant Mg nutrition is a great challenge. Although there have been no reports on the utilization rate of Mg nutrition in tea

plants, different rates of Mg supply in soil can be evaluated using selectively extraction. Overall, individual extraction methods based on the relationship between chemical availability and plant response can produce optimal fertilizer recommendations. meaning that CaCl_2 could be recommended for plant-available Mg extraction in tea plantation soil, further predict efficiency of Mg fertilizer application in tea plantations.

Effects of Mg fertilization on tea quality

a high-quality green tea is characterized by high amino acids and appropriate polyphenols content, since they are the principal contributors to the taste of freshness and bitterness of green tea, respectively (Wang et al. 1988; Zhang et al. 2016). Previous studies have shown that Mg nutrition plays an important role in the yield and quality formation of tea (Ruan et al. 1998; Ruan et al. 1999; Ruan et al. 2012). It was found that a higher levels of Mg supply are beneficial to the accumulation of amino acids and the long-distance transportation of sugars in xylem and phloem of tea plants (Ruan et al. 2012). In this study, the application of Mg fertilizer had a great effect on decreasing the ratio of total polyphenol to amino acid in the tea leaves (Figure 3), resulting in the increase in tea quality (Zhang et al. 2016). This can be attributed to the promotion of Mg on N fixation and metabolism in plants (Fischer et al. 1998; Ruan et al. 1998). In addition, the effects of Mg nutrition on the synthesis of secondary metabolites has also been widely studied, such as the relationship between Mg deficiency in plants and photooxidation (Tewari et al. 2006). Flavonoids, the main secondary metabolites in tea plant, have also been proven to be involved in photoprotection (Cakmak and Kirkby, 2008), the interaction mechanisms of Mg and flavonoids are worth further study. Previous study reported that the share of the total Mg bound to chlorophyll ranges from about 6% to 25 % and highest values are observed in Mg-deficient plants (Marschner, 2012). In this study, a weak correlation was observed between chlorophyll (chl_a, chl_b) and soil Ex-Mg concentration. Indicating that it's not the Mg bound to chlorophyll that is limiting its synthesis under Mg-deficient conditions (Jóska et al. 2013).

Mg fertilizer use efficiency and soil's contribution to plant availability of Mg

Most nutrients in soil cannot be directly absorbed and utilized by plants. Papenfuß and Schlichting (1979) suggested that the fixed Mg pool in soil can substantially contribute to plant Mg nutrition. However, inconsistent results on the contribution of the fixed soil Mg pool to plant Mg nutrition was also reported (Christenson and Doll, 1973; Kidson et al. 1975; Salmon and Arnold, 1963). It is worth noting that the ratio of polyphenols to amino acids highly correlated with the total Mg in the soil with the +Mg group (Figure 4a), while it was uncorrelated with Ex-Mg (Figure 4b). In addition, the Mg fertilizer also showed higher use efficiency in soil with low total Mg (Figure 5a). Concerning a poor relationship always be reported between the plant growth response and extractable nutrients in the soil (Gransee and Führs, 2013; Ortas et al. 1999) as we have discussed earlier, these results suggested that the desorption and

mineralization of non-active Mg in soil may have potential effect on the plant availability of the Mg fertilizer. However, the mineralization of non-active Mg in soil always was affected by many other factors, such as soil texture. In general, compare to Ex-Mg, the total Mg shown a higher correlation to plant availability (including uptake and quality-improvement) of Mg application. This may also be attributed to that a clay with Mg deficiency could absorb more Mg than a clay with Mg adequacy. The latter will easily make more Mg available for plant uptake. However, the new knowledge should be confirmed by further study on transformation in soil and metabolism in plant.

Fertilizers have higher plant availability because they are usually manufactured as a form that can be directly absorbed and utilized by plant. However, plant availability can be affected by soil background nutrient. For example, plant availability of soil Mg and other cations also related to the cation activity ratios in the soil solution. It was found that the approximate critical activity ratio for Mg/K (molar basis) should be 0.5 for good growth (Beckett, 1972). A positive effect of Mg on N uptake efficiency has been well reviewed by Witold (2013). In tea plantation, a previous study found that the yield and quality improvement effects of Mg application varied greatly in soils with different N levels (Ruan et al. 2012). In this study, compared to soils containing high N, soils with low N feature a higher plant availability of Mg fertilizers (Figure 5b). Moreover, there was significant and negative correlations between the ratio of polyphenol to amino acids and the total N contents of soil in the control group, while uncorrelation was observed in the experimental group (Figure 4c, e), suggesting that the application of Mg fertilizer may improve the utilization rate of N. The optimal yield forming effect of fertilizer Mg can generally occur under conditions of relatively low N supply, but high supply of Mg.

The content of Ex-Mg from different tea-producing areas varied greatly (30.71 - 245.04 mg kg⁻¹). However, high consumption of Mg has been observed by comparing the status of Mg pre- and post- tea cultivation in soil, indicated that Mg depletion in tea soils should be a growing concern for tea production, although the potting system used in this experiment enlarged the nutrient cycle and Mg consumption. Moreover, acidic soil (pH ranged from 3.59 to 4.93) is not conducive to the absorption and utilization of Mg nutrition (Senbayram et al. 2016). In order to avoid situations of field scale Mg deficiency, precise knowledge of critical threshold values for Mg application in the tea plantation is required. In this study, the proportion of Mg taken up in plant tissue from the Mg supply were 1.30 %, 10.17 %, and 3.84 %, in root, stem and leaf respectively (Table 3). In cases Mg fertilizer use efficiency is the only consideration (the harvest part of 3.84%), about 6.5 kg Mg fertilizer is needed for 100 kg tea leaves (about 2.5mg/g Mg) harvest (base on the soil nutrient balance). However, we also found that Mg, which almost 4 times of the harvest amount (15.31 / 3.84), can be absorbed and utilized by plants and stored in roots and stems. Considering reuse of storage nutrient are frequently occurred in tea plants (Fan et al. 2019), and Mg always preferential distribution to new shoots than old tissue, as well as the application of pruning litter can maintain plant biomass stability and soil nutrient supplement, our results show that extreme Mg deficiencies (base on both nutrient balance and tea quality) can be effectively avoided in tea plantation by application of about 2-3 kg Mg year⁻¹ (100 kg leaves harvest)⁻¹.

Conclusion

The plant availability of Mg fertilizer (6.08 % - 29.56 %) was quantified for the first time through a comparison of the uptake and quality-improvement effect in tea soils from 12 typical plantations in China. Moreover, the concentrations of exchangeable Mg, especially extracted by calcium chloride (CaCl_2), highly correlated with the utilization of tea plant. In addition, higher plant availability and quality-improvement effects (decreased the ratio of total polyphenol to amino acid) were observed in soils with low level of total Mg content, as well as N deficiency status. Our results provide a potential theoretical for guidance of Mg fertilization in tea plantations.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Figure S1. Tea plant and experimental design.

Figure S2. Appearance of Soil from tea plantations at 12 countries in china after plant harvest.

Table S1. Information of soil samples from 12 tea plantations in China.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

QFZ, DDT and XYY gathered samples, MYL participated in the study design, QFZ, SPG, YH, YPC and KN performed data analysis, QFZ and XDY interpreted the results and drafted the manuscript. JYR conceived of the study, provided funding, and gave guidance on experimental design. All authors read and approved the final manuscript.

Funding

The work was supported by the Central Public-interest Scientific Institution Basal Research Fund (Y2020PT08), the Earmarked Fund for China Agriculture Research System Ministry of Agriculture of China (CARS19), and the Chinese Academy of Agricultural Sciences through Agricultural Sciences Innovation Project (CAAS-ASTIP-2017-TRICAAS).

References

1. Beckett P (1972) Critical cation activity ratios. *Adv. Agron.* 24:379.
2. Cakmak I (2013) Magnesium in crop production, food quality and human health. *Plant Soil* 368: 1–4.
3. Cakmak I, Kirkby EA (2008) Role of magnesium in carbon partitioning and alleviating photooxidative damage. *Physiol Plant* 133:692-704
4. Cakmak I, Yazici AM (2010) Magnesium: a forgotten element in crop production. *Better Crops* 94:23-25
5. Christenson DR, Doll EC (1973) Release of magnesium from soil clay and silt fractions during cropping. *Soil Sci* 116:59–63
6. Conn SJ, Conn V, Tyerman SD, Kaiser BN, Leigh RA, Gilliam M (2011) Magnesium transporters, MGT2/MRS2-1 and MGT3/MRS2-5, are important for magnesium partitioning within *Arabidopsis thaliana* mesophyll vacuoles. *New Phytol* 190:583-594
7. Deng W, Luo KM, Li DM, Zheng XL, Wei XY, Smith W, Thammina C, Lu LT, Li L, Pei Y (2006) Overexpression of an *Arabidopsis* magnesium transport gene, AtMGT1, in *Nicotiana benthamiana* confers Al tolerance. *J Exp Bot* 57:4235-4243
8. Dechen AR, Carmello QAC, Monteiro FA, Nogueirol RC (2015) Role of magnesium in food production: An overview. *Crop Pasture Sci* 66: 1213–1218.
9. Dragicevic V, Mladenovic-Drinic S, Stojiljkovic M, Filipovic M, Nikolic B, Babic V, Kravic N (2016) Maize inbreds from different heterotic groups as favorable sources for increased potential bioavailability of magnesium, iron, manganese and zinc. *CHIL J AGR RES* 76:213-219
10. Fan K, Zhang Q, Tang D (2020) Dynamics of nitrogen translocation from mature leaves to new shoots and related gene expression during spring shoots development in tea plants (*Camellia sinensis* L.). *Journal of Plant Nutrition and Soil Science* 183(2)
11. Farhat N, Rabhi M, Falleh H, Falleh, K, Smaoui A, Abdely C, Lachaâl M, Karray-Bouraoui N (2013) Interactive effects of excessive potassium and Mg deficiency on safflower. *Acta Physiol Plant* 35:2737-2745
12. Fischer E, Lohaus G, Heineke D, Heldt H (1998) Magnesium deficiency results in accumulation of carbohydrates and amino acids in source and sink leaves of spinach. *Physiol Plant* 102:16-20
13. Gerendás J, Führs H (2013) The significance of magnesium for crop quality. *Plant Soil* 368:101-128
14. Gransee A, Führs H (2013) Magnesium mobility in soils as a challenge for soil and plant analysis, magnesium fertilization and root uptake under adverse growth conditions. *Plant Soil* 368:5-21
15. Grzebisz W, Przygocka-Cyna K, Szczepaniak W, Diatta J, Potarzycki J (2010) Magnesium as a nutritional tool of nitrogen management – Plant production and environment. *Journal of Elementology* 15: 771–788.
16. Guo WL, Nazim H, Liang ZS, Yang DF (2016) Magnesium deficiency in plants: an urgent problem. *Crop J* 4:83-91

17. Hariadi, Y., and Shabala, S. (2004). Screening broad beans (*Vicia faba*) for magnesium deficiency. I. Growth characteristics, visual deficiency symptomatology and plant nutritional status. *Funct. Plant Biol.* 31, 529–537. doi: 10.1071/fp03201
18. Iatrou M, Papadopoulos A, Papadopoulos F, Dichala O, Psoma P, Bountla A (2015) Determination of Soil-Available Micronutrients using the DTPA and Mehlich 3 Methods for Greek Soils Having Variable Amounts of Calcium Carbonate. *COMMUN SOIL SCI PLAN* 46:1905-1912
19. Jóska G, Hendrik F (2013) The significance of magnesium for crop quality. *Plant Soil* 368:101–128
20. Kopittke PM, Menzies NW (2007) A review of the use of the Basic Cation Saturation Ratio and the 'ideal' soil. *SSSAJ* 71:259–265
21. Kidson EB, Hole FA, Metson AJ (1975) Magnesium in New Zealand soils III. Availability of non-exchangeable magnesium to white clover during exhaustive cropping in a pot experiment. *N Z J Agric Res* 18:337–349
22. Lasa B, Frechilla S, Aleu M, González-Moro B, Lamsfus C, Aparicio-Tejo P (2000) Effects of low and high levels of magnesium on the response of sunflower plants grown with ammonium and nitrate. *Plant Soil* 225:167-174
23. Marschner H (2012) Mineral nutrition of higher plants. Academic, Elsevier
24. Mayland HF, Wilkinson SR (1989) Soil factors affecting magnesium availability in plant-animal systems: a review. *J. Anim. Sci* 67: 3437–3444
25. Mehlich A (1984) Mehlich 3 soil test extractant. A modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis* 29: 1409–1416
26. Mikkelsen R (2010). Soil and fertilizer magnesium. *Better Crops* 94: 26–28
27. Nelson WL, Mehlich A, Winters E (1953) The development, evaluation and use of soil tests for phosphorus availability. In *Soil and Fertilizer Phosphorus*; Pierre, W.E. and Nornan, A.G. (eds.); American Society of Agronomy: Madison, Wisc., 153–188
28. Nelson WE, and JB, Jones JR (1972) Evaluation of magnesium soil testing methods. In: J. B. Jones, Jr., M. C. Blount and S. R. Wilkinson (Ed.) *Magnesium in the Environment: Soils. Crops. Animals and Man.* pp217-225. Taylor County Publ. Co.. Reynolds, GA
29. Ni K, Liao WY, Yi XY, Niu SY, Ma LF, Shi YZ, Zhang QF, Liu MY, Ruan JY (2019) Fertilization status and reduction potential in tea gardens of China. *J Plant Nutr* 25: 421–432
30. Oenema O, Kros H, de Vries W (2003) Approaches and uncertainties in nutrient budgets: Implications for nutrient management and environmental policies. *European Journal of Agronomy* 20: 3–16
31. Ortas I, Güzel N, Ibrikçi H (1999) Determination of potassium and magnesium of soils using different soil extraction procedures in the upper part of mesopotamia (in the Harran plain). *Commun Soil Sci Plant Anal* 30:2607–2625
32. Papenfuß KH, Schlichting E (1979) Bestimmende Faktoren des Mg-Haushaltes von Böden in der Bundesrepublik Deutschland. *Magnes Bull* 1:12–14

33. Pol F, Traore B (1993) Soil nutrient depletion by agricultural production in Southern Mali Fert. Res. 36: 79–90
34. Ruan JY, Wu X, Ye Y, Hardter R (1998) Effect of potassium, magnesium and sulphur applied in different forms of fertilisers on free amino acid content in leaves of tea (*Camellia sinensis* L.). J Sci Food Agric 76:389-396
35. Ruan JY, Wu X, Hardter R (1999) Effects of potassium and magnesium nutrition on the quality components of different types of tea. J Sci Food Agric 79:47-52
36. Ruan JY, Guan YL, Wu X (2002) Status of Mg Availability and the Effects of Mg Application in Tea Fields of Red Soil Area in China. Scientia Agricultura Sinica. 35:815 -820
37. Ruan J, Zhang F, Wong MH (2000) Effect of nitrogen form and phosphorus source on the growth, nutrient uptake and rhizosphere soil property of *Camellia sinensis* L. Plant and Soil 223: 65-73
38. Ruan J, Ma L, Shi Y, Zhang F (2004) Effects of litter incorporation and nitrogen fertilization on the contents of extractable aluminium in the rhizosphere soil of tea plant (*Camellia sinensis* (L.) O. Kuntze). Plant and soil 26: 283-296
39. Ruan JY, Ma LF, Yang YJ (2012) Magnesium nutrition on accumulation and transport of amino acids in tea plants. J Sci Food Agric 92:1375-1383
40. Ruan JY, Gerendas J (2015) Absorption of foliar-applied urea-N-15 and the impact of low nitrogen, potassium, magnesium and sulfur nutritional status in tea (*Camellia sinensis* L.) plants. J. Soil Sci. Plant Nutr 61:653-663
41. Salmon BC, Arnold PW (1963) The magnesium uptake under exhaustive cropping. J Agric Sci 61:421–425
42. Scheffer F, Schachtschabel P (2002). Lehrbuch der Bodenkunde (Heidelberg:Spektrum Akademischer Verlag)
43. Senbayram M, Gransee A, Wahle V, Thiel H (2016) Role of magnesium fertilisers in agriculture: plant–soil continuum. Crop and Pasture Science 66:1219-1229
44. Staugaitis G, Rutkauskienė (2010) Comparison of magnesium determination methods as influenced by soil properties. Agriculture (Žemdirbystė) 97:105–116
45. Soremi AO, Adetunji MT, Adejuyigbe CO, Bodunde JG, Azeez JO (2017) Effects of poultry manure on some soil chemical properties and nutrient bioavailability to soybean. J. agric. ecol. res. int:1-10
46. Tran TS, Giroux M, Guilbeaut J, Audess P (1990) Evaluation of Mehlich III extractant to estimate the available P in Quebec soils. Communications in Soil Science and Plant Analysis 21: 1–28
47. Tewari RK, Kumar P, Sharma PN (2006) Magnesium deficiency induced oxidative stress and antioxidant responses in mulberry plants. Sci Hortic 108:7-14
48. Van Raij B, Quaggio JA, da Silva NM (1986) Extraction of phosphorus, potassium, calcium, and magnesium from soils by an ion-exchange resin procedure. Communications in Soil Science and Plant Analysis 17: 547–566.

49. Van Raij (1988) Bioavailable tests: Alternatives to standard soil extractions. *Communications in Soil Science and Plant Analysis* 29: 1553–1570.
50. Wang Y, Chen, Q Ruan, Y, Liu W, Zhu H (1988) Discussion on the chemical standards on quality of Chinese roasted green tea. *J. Tea Sci.* 8: 13-20.
51. Wang, Z., Hassan, M. U., Nadeem, F., Wu, L., Zhang, F., & Li, X. (2020). Magnesium Fertilization improves crop yield in most production systems: a meta-analysis. *Frontiers in Plant Science*, 10, 1727
52. Witold G (2013) Crop response to magnesium fertilization as affected by nitrogen supply. *Plant Soil* 368:23–39
53. Wilkinson S, Welch R, Mayland H, Grunes D (1990) Magnesium in plants: uptake, distribution, function and utilization by man and animals. *Met. Ions Biol. Syst* 26: 33–56
54. Wu X, Ruan JY (1994) Potassium, magnesium and sulphur status of tea gardens in China—Effects of K, Mg and S fertilizers. *Potash Review* 3:1-8
55. Yang XD, Ni K, Ruan JY (2018) Effects of long-term nitrogen application on soil acidification and solution chemistry of a tea plantation in China. *Agriculture Ecosystems & Environment* 252:74-82
56. Yousaf B, Liu GJ, Wang RW, Zia-ur-Rehman M, Rizwan MS, Imtiaz M, Murtaza G, Shakoor A (2016) Investigating the potential influence of biochar and traditional organic amendments on the bioavailability and transfer of Cd in the soil–plant system. *Environ. Earth Sci* 75:374
57. Zbíral J (2016) Determination of plant-available micronutrients by the Mehlich 3 soil extractant—a proposal of critical values. *Plant Soil Environ* 62:527-531
58. Zhang QF, Ruan J (2016) Tea: Analysis and Tasting. In: Caballero B, Finglas PM, Toldrá F (eds) *Encyclopedia of Food and Health*. Academic Press, Oxford, pp 256-267
59. Zhang QF, Tang DD, Liu MY, Ruan JY (2018) Integrated analyses of the transcriptome and metabolome of the leaves of albino tea cultivars reveal coordinated regulation of the carbon and nitrogen metabolism. *Sci Hortic* 231:272-281

Tables

Table 1 Soil properties of tea plantations at the selected 12 countries in China.

| Site | pH | C (mg/g) | N (mg/g) | *soil particle-size distribution (%) | | | soil texture |
|-------------------------|------|-------------|-------------|---|-------|------------|--------------------|
| | | | | clay | silt | total sand | |
| ChengDu (CD) | 3.78 | 112.77 | 11.12 | 15.54 | 65.88 | 18.58 | silty loam |
| GuiLing (GL) | 4.25 | 121.64 | 10.41 | 57.73 | 24.20 | 18.06 | clay |
| HuangGang (HG) | 3.58 | 215.03 | 22.20 | 14.25 | 59.54 | 26.22 | silty loam |
| XinYang (XY) | 4.73 | 120.04 | 11.63 | 27.58 | 55.71 | 16.32 | silty clay loam |
| XiShuangBanNa (XSBN) | 4.93 | 224.05 | 16.40 | 1.63 | 56.94 | 40.81 | silty loam |
| WuXi (WX) | 3.84 | 126.56 | 10.90 | 53.95 | 20.74 | 25.30 | clay |
| YiChang (YC) | 4.64 | 158.27 | 14.52 | 16.23 | 61.70 | 22.06 | silty loam |
| ChangSha (CS) | 4.04 | 136.02 | 14.01 | 0.81 | 38.14 | 60.46 | sandy loam |
| NingDe (ND) | 3.76 | 122.60 | 12.70 | 48.27 | 42.03 | 9.37 | clay |
| QingYuan (QY) | 4.78 | 151.70 | 16.14 | 39.58 | 48.03 | 12.39 | silty clay |
| XiangXi (XX) | 4.47 | 257.43 | 24.50 | 45.82 | 39.45 | 14.73 | clay |
| ChongQing (CQ) | 3.84 | 114.65 | 11.52 | 42.76 | 41.69 | 15.54 | silty clay |

*clay($\leq 2 \mu\text{m}$), silt (2-50 μm), total sand (50-2000 μm).

Table 2 Pearson correlation coefficients between soil exchangeable Mg and Mg concentrations in tea plants (n=36)

| Soil | Extract | Root Mg | Stem Mg | Leaf Mg | chl _a | chl _b |
|---------------------------------------|---------------------|----------|----------|----------|------------------|------------------|
| Initial | Mehlich-3 | 0.535** | 0.638*** | 0.680*** | 0.647*** | 0.444** |
| | NH ₄ OAC | 0.406* | 0.607*** | 0.640*** | 0.573*** | 0.343* |
| | CaCl ₂ | 0.536** | 0.689*** | 0.661*** | 0.409* | 0.178 |
| | BaCl ₂ | 0.526** | 0.684*** | 0.698*** | 0.571*** | 0.357* |
| Post cultivation | Mehlich-3 | 0.499** | 0.669*** | 0.860*** | 0.314 | 0.176 |
| <i>CK group</i> | NH ₄ OAC | 0.587** | 0.707*** | 0.866*** | 0.322 | 0.166 |
| | CaCl ₂ | 0.636*** | 0.733*** | 0.906*** | 0.352* | 0.184 |
| | BaCl ₂ | 0.625*** | 0.679*** | 0.881*** | 0.389* | 0.245 |
| <i>Mg group</i> | Mehlich-3 | 0.447** | 0.621*** | 0.762*** | 0.409* | 0.280 |
| | NH ₄ OAC | 0.562*** | 0.547*** | 0.632*** | 0.400* | 0.312 |
| | CaCl ₂ | 0.650*** | 0.640*** | 0.740*** | 0.531** | 0.383 |
| | BaCl ₂ | 0.604*** | 0.613*** | 0.728*** | 0.473** | 0.359* |
| DMg change | Mehlich-3 | -0.428* | -0.475** | -0.413* | -0.713*** | -0.515** |
| (Post cultivation CK Group - Initial) | NH ₄ OAC | 0.132 | -0.0326 | 0.108 | -0.435** | -0.292 |
| | CaCl ₂ | 0.628*** | 0.702*** | 0.917*** | 0.317 | 0.175 |
| | BaCl ₂ | 0.265 | 0.0666 | 0.401* | -0.255 | -0.156 |

*, p<0.05; **, p<0.01; ***, p<0.001

Table 3. Magnesium fertilizer use efficiency in typical tea plantation soils

| Site | Mg fertilizer use efficiency in tea plant (%) | | | |
|----------------------|---|-------|------|-------|
| | Root | Stem | Leaf | Total |
| ChengDu (CD) | 0.01 | 12.32 | 4.40 | 16.73 |
| GuiLing (GL) | 0.56 | 11.52 | 0.32 | 12.40 |
| HuangGang (HG) | 1.04 | 5.52 | 5.00 | 11.56 |
| XinYang (XY) | 0.01 | 11.48 | 0.24 | 11.73 |
| XiShuangBanNa (XSBN) | 1.84 | 10.60 | 4.16 | 16.60 |
| WuXi (WX) | 0.56 | 25.80 | 3.20 | 29.56 |
| YiChang (YC) | 2.16 | 1.00 | 2.92 | 6.08 |
| ChangSha (CS) | 0.88 | 4.80 | 5.00 | 10.68 |
| NingDe (ND) | 1.08 | 9.60 | 6.20 | 16.88 |
| QingYuan (QY) | 2.60 | 11.28 | 4.68 | 18.56 |
| XiangXi (XX) | 0.52 | 7.04 | 2.88 | 10.44 |
| ChongQing (CQ) | 4.28 | 11.12 | 7.12 | 22.52 |
| Mean value | 1.30 | 10.17 | 3.84 | 15.31 |

Figures

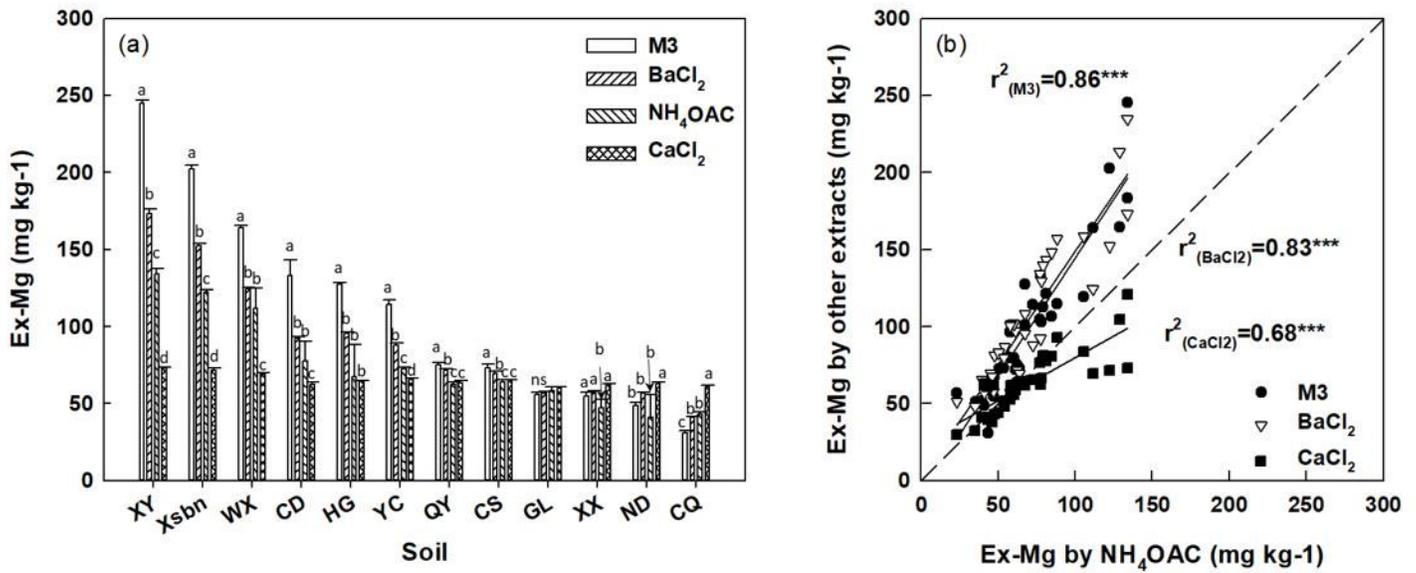


Figure 1

Contents of exchangeable Mg (means \pm SD, $n=3$) extracted by 1.0 mol L⁻¹ CaCl₂, 0.01 mol L⁻¹ BaCl₂, Mehlich 3 and 1.0 mol L⁻¹ NH₄.OAc in soils pre - tea cultivation (a), and the correlation of exchangeable Mg extracted by different extracts (b). Different letters above the bar indicate significant ($p < 0.01$) differences among different extraction.

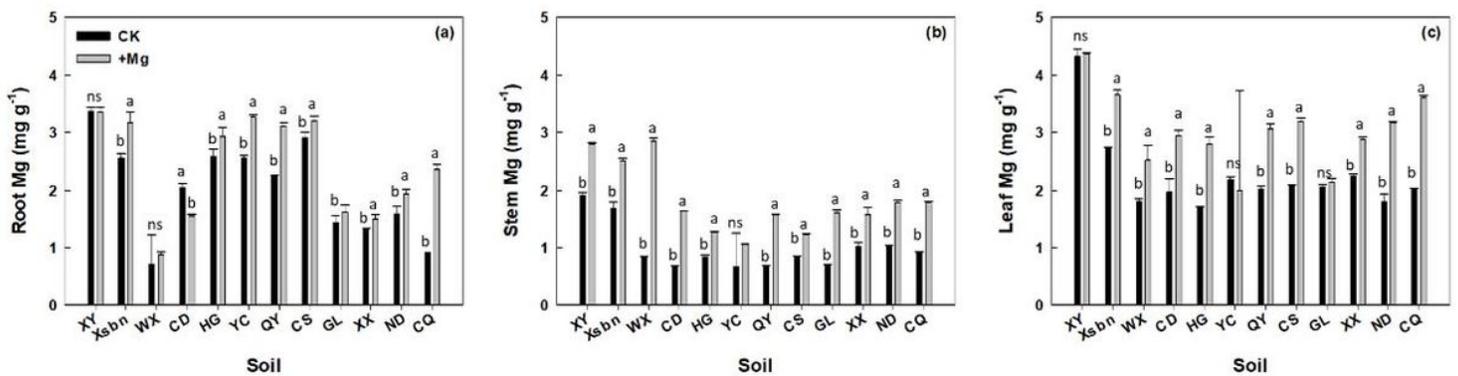


Figure 2

Concentration of magnesium (means \pm SD, $n=3$) in root (a), Stem (b) and Leaf (c) of tea plants. "ns" and different letters above the bar indicate insignificant and significant ($p < 0.01$) differences, respectively, between two magnesium treatments.

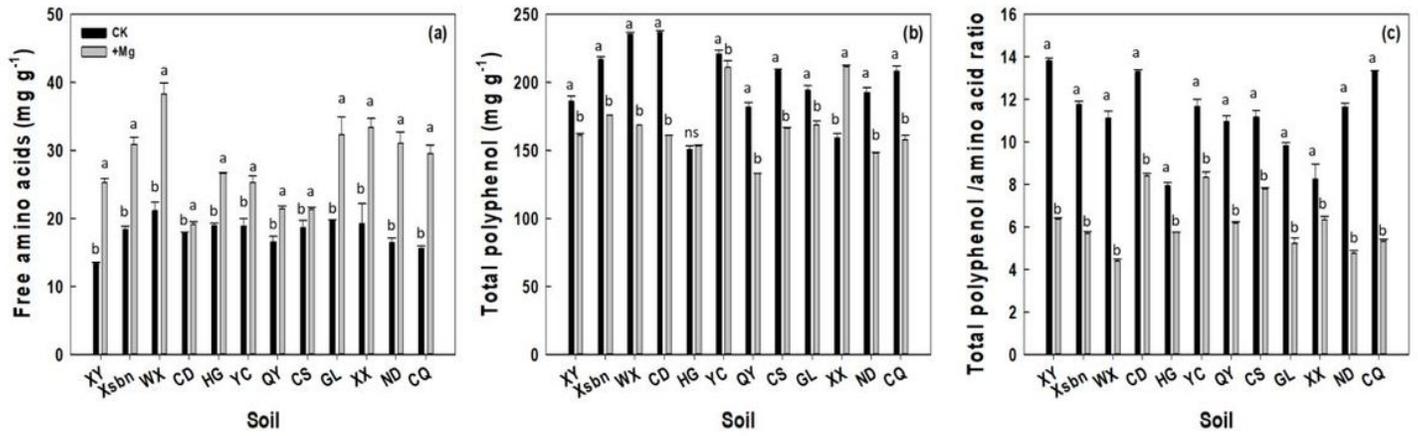


Figure 3

Effect of Mg supply on concentrations of free amino acid (a), polyphenol (b) and polyphenol/free amino acid (TP/AA) ratio (c) in tea leaf under Mg application (means \pm SD, $n=3$). "ns" and different letters above the bar indicate insignificant and significant ($p < 0.01$) differences, respectively, between two magnesium treatments.

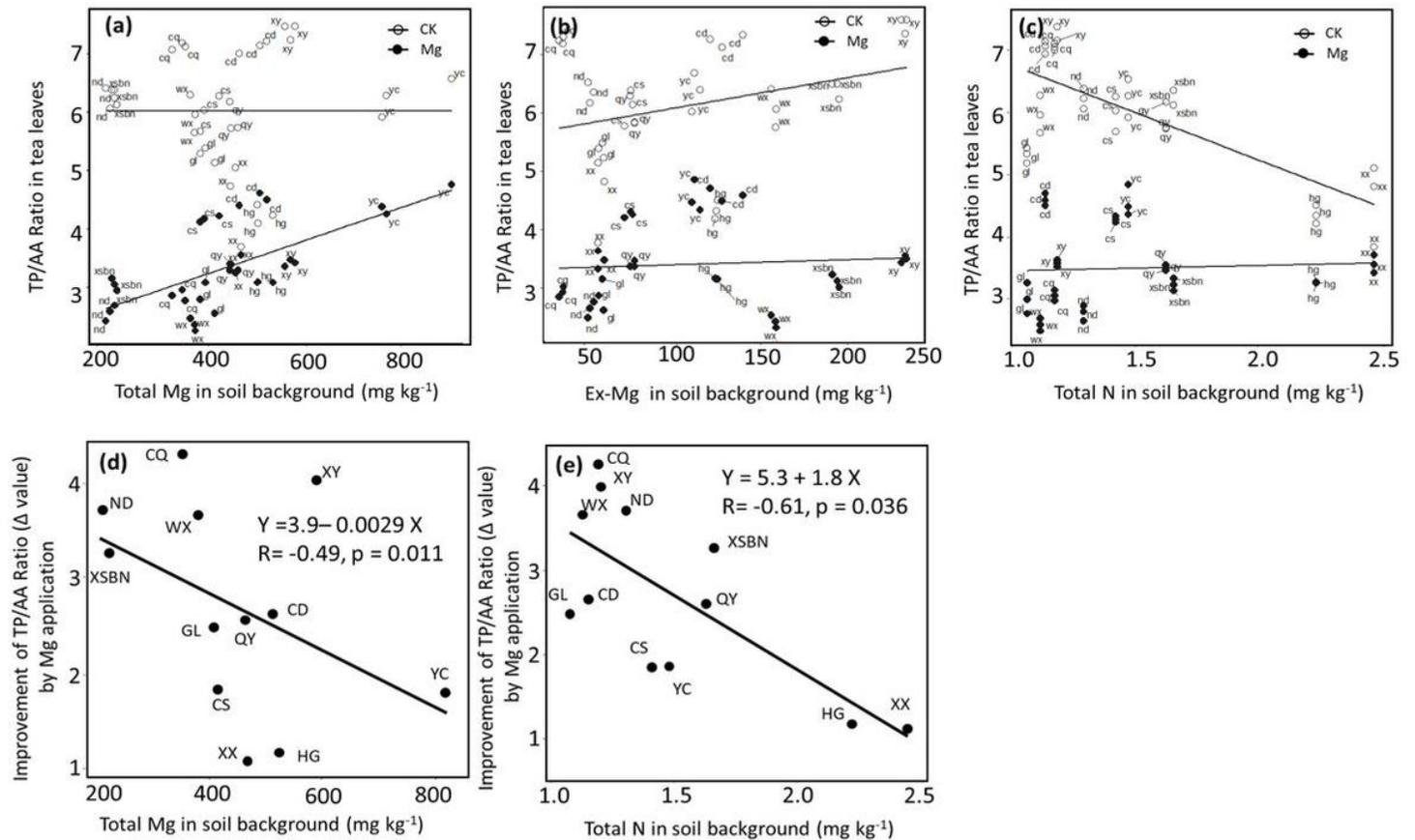


Figure 4

Quality changed in tea leaves after magnesium (Mg) application: The correlation between polyphenol/free amino acid (TP/AA) ratio (a, b, c) or change of TP/AA ratio (Δ value) pre- and post- tea plant cultivation (d, e) in tea leaves and content of total Mg (a, d), exchangeable Mg (b) or total nitrogen (e) in soil. Total Mg was extracted with nitric acid, perchloric acid and hydrofluoric acid from soil.

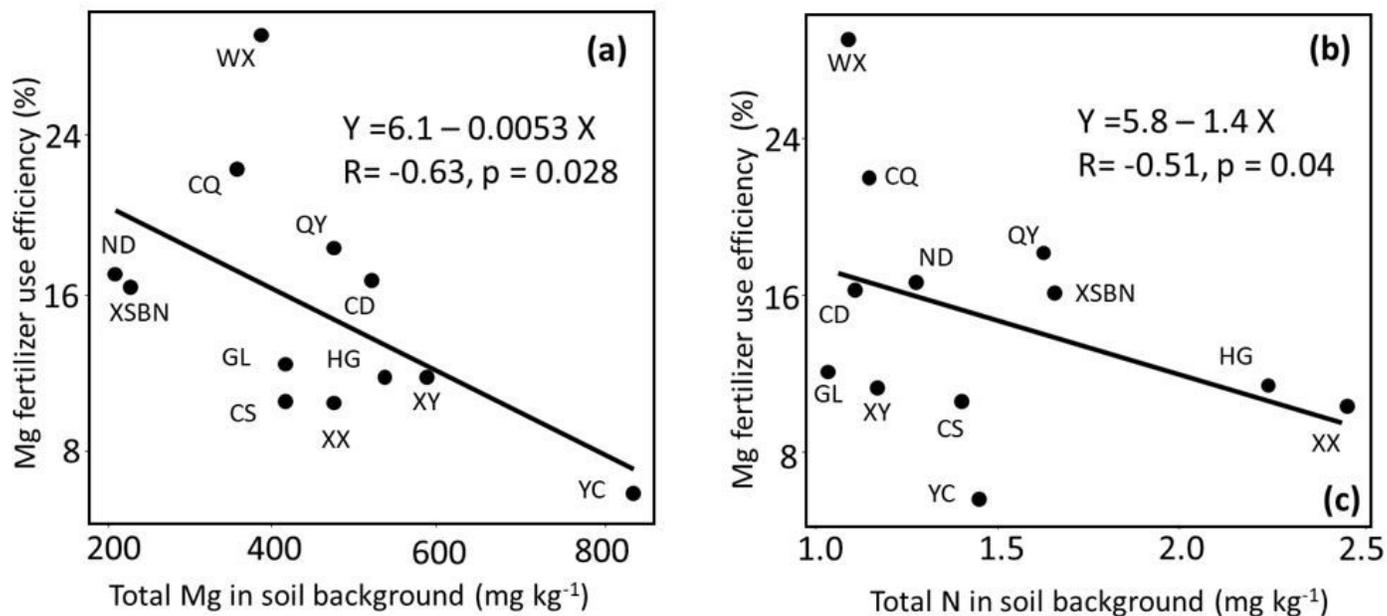


Figure 5

The correlation between content of total magnesium (a) or nitrogen (b) in soil and magnesium fertilizer use efficiency. Total Mg was extracted with nitric acid, perchloric acid and hydrofluoric acid from soil.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [FigureS13.docx](#)
- [TableS1.docx](#)