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REGULAR ARTICLE

Magnesium isotope fractionation reflects plant response to magnesium deficiency in magnesium uptake and allocation: a greenhouse study with wheat

Yi Wang · Bei Wu · Anne E. Berns · Ying Xing · Arnd J. Kuhn · Wulf Amelung

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Abstract

Aims Magnesium (Mg) deficiency is detrimental to plant growth. However, how plants respond to Mg deficiency via regulation of Mg uptake and allocation is yet not fully understood. In this study, we tested whether Mg isotope compositions (δ^{26}Mg) associated with Mg mass balance of the plants could be used as an indicator to trace Mg uptake and subsequent translocation processes under sufficient and low-Mg supply conditions. We aimed at using stable isotope fractionation as a novel proxy for nutrient uptake and cycling in plants.

Methods We grew wheat plants (*Triticum aestivum*) in a greenhouse under control (1 mM Mg) and low-Mg supply (0.05 mM Mg) conditions, respectively. The Mg concentrations and isotope compositions in roots, stems, leaves and spikes/grains at different growth stages were analyzed.

Results Wheat plants were systematically enriched in heavy Mg isotopes relative to the nutrient solution regardless of Mg supply conditions. With crop growth, the δ^{26}Mg of the whole plants, as well as each plant organ, gradually shifted towards higher values in the control. However, the δ^{26}Mg value of the whole plants in the low-Mg supply did not vary significantly. In addition, the wheat stems and spikes showed continuous enrichment of lighter Mg isotopes in the low-Mg supply than those in the control.

Conclusions As reflected from Mg isotope compositions, the Mg supply in the growth media could affect the Mg uptake and subsequent translocation processes in plants. Changes in δ^{26}Mg indicated that wheat plants likely regulated their Mg uptake strategy by switching between active and passive pathways during their life cycle. When Mg supply was low, a more negative δ^{26}Mg value of the spikes suggested a potentially enhanced remobilization of Mg from leaves to spikes. Our results showed that Mg stable isotopes can provide new insights into plants' response to nutrient shortage.

Keywords Mg deficiency · Low-Mg supply · Mg isotope compositions (δ^{26}Mg) · Root uptake · Root-shoot transport · Mg remobilization

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Abbreviations

ICP-MS inductively coupled plasma mass spectrometry

Introduction

Magnesium (Mg) is one of the essential macronutrients for plants, playing a key role in many biochemical processes, especially in photosynthesis as the central atom in chlorophyll (Maguire and Cowan 2002; Willows 2007). Moreover, Mg is necessary for a series of enzyme activities and for protein synthesis, functioning as a bridge element between enzymes and substrates, for example, in the synthesis of ATP (Marschner 2011; Shaul 2002). Recent studies have also revealed a role of Mg in alleviating negative effects under adverse growth conditions, such as aluminum and manganese toxicity (Bose et al. 2011; Gransee and Führs 2013), photooxidative damage (Cakmak and Kirkby 2008), and heat stress (Mengutay et al. 2013). Therefore, Mg availability may significantly affect both productivity and quality of agricultural and horticultural crops (Gransee and Führs 2013; Hermans et al. 2005). Nevertheless, Mg deficiency is increasingly observed worldwide in forest and agricultural ecosystems, particularly in soils intensively fertilized with K^+ and NH_4^+, or in soils with low pH due to the potential loss of Mg by leaching (Cakmak and Yazici 2010; Guo et al. 2016).

When Mg is not supplied in sufficient amounts, a series of Mg-deficiency symptoms (e.g. interveinal chlorosis) appear: the photosynthetic rate and the export of sucrose from leaves via phloem decreases, leading to accumulation of carbohydrates in source leaves and growth inhibition of sink organs (Verbruggen and Hermans 2013). In order to obtain and preserve high contents of Mg in plants, in particular when Mg supplies are limited, plants have developed efficient physiological systems for Mg uptake and internal translocation (Hermans et al. 2013). For example, the MGT/MRS2 family of Mg transporters has been identified as the primary Mg transporters playing a key role in active Mg uptake by plants, e.g. *Arabidopsis*, rice and maize (Gebert et al. 2009; Li et al. 2001, 2016; Saito et al. 2013). Transport of Mg assisted with these transporters is considered as an active pathway which consumes energy and requires Mg firstly to be bound with membrane proteins during the cross-membrane transport of Mg against concentration gradient (Chen et al. 2018). On the other hand, Mg is also thought to enter into root cells via passive pathways such as non-selective cation channels (Demidchik and Maathuis 2007; Ogura et al. 2018). In this case, Mg moves into the cells with the water stream driven by energy-free diffusion along concentration gradient (Hermans et al. 2013). Both the active and passive pathways can contribute to the root uptake of Mg during plant growth. However, which pathway dominates in the Mg uptake processes regulated by plants, especially when plants are grown under different Mg supply conditions or at different growth stages, still remains unknown.

Recent studies on biotic-induced Mg isotope fractionations have provided new insights into the physiological activities of plants (Schmitt et al. 2012; Teng 2017). Apparent differences in Mg isotope composition have been reported between plants and their environments in previous studies as a result of plant uptake-induced Mg isotope fractionation (e.g. Black et al. 2008; Bolou-Bi et al. 2010, 2012; Opfergelt et al. 2014). Among these studies, Black et al. (2008) and Bolou-Bi et al. (2010) cultivated plants in pot experiments under greenhouse conditions as a model system, and demonstrated that Mg isotope compositions of the mature plants were heavier than those of the nutrient solution. The authors, however, only measured the Mg isotope compositions of plants grown with sufficient Mg supply at maturity stage. The dynamic Mg isotope fractionation during the plant life cycle under different Mg conditions still remains unclear. We hypothesize that when supplied with different amounts of Mg, plants may regulate the Mg uptake through different combinations of the active and passive pathways. This may result in different Mg isotope compositions of the plants. In addition, Mg remobilization from older leaves to younger ones or reproductive organs (e.g. grain) during leaf senescence and grain filling play a more important role in plant growth when Mg is deficient in the growth media (Tiryakioğlu et al. 2014; Maillard et al. 2015; Billard et al. 2016; Peng et al. 2019). As Mg remobilization processes will change Mg isotope compositions of different plant organs (Kimmig et al. 2018), we further hypothesize that variation of Mg isotope compositions of plant organs can reflect Mg remobilization during plant growth.

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Therefore, in the present study we aimed to understand the role of Mg supplies in Mg isotope fractionation in plants, and how plants respond to Mg supplies during plant growth as indicated by variation of isotope compositions. Hence, we studied Mg isotope compositions of different organs of wheat plants grown under greenhouse conditions with either sufficient (control) or low-Mg supply, to elucidate the change of Mg isotope compositions of the plants in relation to varied uptake and remobilization processes of Mg under the two Mg supply conditions at different plant growth stages. We hypothesize that such isotopic data provide information on biological processes during Mg uptake and internal translocation.

Materials and methods

Plant growth and sampling

The seeds of summer wheat (*Triticum aestivum*) were germinated on a moistened filter paper in the dark for 48 h. Afterwards, well developed seedlings were selected and transferred into small pots filled with sterilized quartz sand (Quarzwerke Witterschlick GmbH, Witterschlick, Germany. 0.71–1.25 mm. $SiO_2 > 98\%$, $MgO < 0.1\%$, w.) which had been pre-cleaned thoroughly with deionized water. In the first three days after transfer, the seedlings were watered without nutrient supply to allow them to adapt to the sand growing environment. Afterwards, the seedlings were placed into larger plastic pots containing sterilized quartz sand (2 plants per pot) and cultivated in controlled climatic environments until harvest (day/night rhythm 16/8 h, temperature 24/18 °C, relative humidity 50–60%, and light intensity 250 μmol m^{-2} s^{-1} supplied by fluorescent tubes). A modified ½ Hoagland nutrient solution (Table S1, Online Resource) was used to supply nutrients for plant growth. Compared with sufficient Mg supply (1 mM Mg, the control), 5% of the Mg concentration (0.05 mM Mg) was provided to the plants as the low-Mg supply. Before flowering (46 days after seedling), the nutrient solutions were supplied three times a day (9 am, 1 pm, and 5 pm, respectively) at a rate of 30 mL min^{-1} for one minute each time per pot via a pump controlled by a timer (Fig. S1, Online Resource). After flowering, another 30 mL nutrient solution was applied for the 4th time at 8 pm additionally in order to account for the increased water demand of the plants. To check whether changes of Mg speciation in the nutrient solution might have occurred when varying the Mg concentrations, the chemical equilibrium model Visual MINTEQ v3.0 software was used. The contribution of the deionized water and quartz sand to plant uptake of Mg was also evaluated, respectively (Chap. 1 and 2, Online Resource). Results showed that the sand-released Mg did not interfere the Mg isotope composition of the nutrient solution. For better illustration, the Mg isotope composition of sand-released Mg was not shown in the figures.

To study Mg isotope fractionation during the growth stages, wheat plants were sampled when they were in booting, flowering, post-flowering and at maturity (39, 46, 67 and 82 days after seedling, respectively). Four pot replicates were set for both the control and the low-Mg supply at each sampling time. In the low-Mg supply, due to the premature death of plants in three of the four pots, there was only one pot in which plants were still alive and sampled at maturity stage without replicates. Upon sampling, the two wheat plants in each pot were carefully taken out together with the sand which was

then washed off with running deionized water. The two wheat plants were then separated into roots, stems, leaves, and spikes and the respective organs of both two plants were pooled into one replicate per pot. At the stage of maturity, grains were isolated from their bran instead of full spikes as done in the previous stages. All plant samples were lyophilized and milled to powder in a custom-designed ball mill (Shaker Viba 330, Collomix GmbH, Germany) using metal-free plastic bottles and tungsten carbide milling balls.

Sample preparation, Magnesium purification and isotope analysis

About 50 mg plant materials were weighed in Teflon tubes and digested in a mixture of 3 ml ultrapure HNO_3 (68%) and 1 ml H_2O_2 (30%, p.a.) in a pressurized microwave-assisted acid digestion system (turboWAVE® Inert, Milestone Srl, Italy). Elemental concentrations were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7900, Germany).

The Mg purification procedures were performed in a customer-designed laminar flow box in a cleanroom at the Agrosphere Institute at Forschungszentrum Jülich GmbH. An aliquot of digested solution was evaporated to dryness on a hot plate at 90 °C. The dried sample material was re-dissolved in 2 ml 1 M ultrapure HNO_3. The separation of Mg from matrix elements was carried out by cation exchange chromatography using modified procedures of Teng et al. (2007) and Wombacher et al. (2009) (Table S2, Online Resource). Full recovery of Mg (> 95%) and the absence of matrix elements were validated by analyzing their concentrations in the eluted Mg solution. Magnesium isotope ratios were measured on a multi-collector ICP-MS (MC-ICP-MS, Nu Plasma II, Nu Instruments Ltd., UK). To correct instrumental mass bias, a strategy of standard-sample-standard bracketing was applied with matched Mg concentrations between the standard and the samples (difference < 5%). Magnesium isotope standard NIST SRM 980 was used as the bracketing standard in the present work. Therefore, the measured Mg isotope ratios of a sample were then converted to the δ notation as follows:

$$\delta^{X}\mathrm{Mg}\ (‰) = \left[\frac{\left({}^{X}\mathrm{Mg}/{}^{24}\mathrm{Mg}\right)_{\mathrm{sample}}}{\left({}^{X}\mathrm{Mg}/{}^{24}\mathrm{Mg}\right)_{\mathrm{NIST980}}} - 1\right] \times 100 \quad (1)$$

where x represents 25 or 26. The three-isotope-plot, generated by the measured δ^{26}Mg and δ^{25}Mg values, indicated the absence of mass-independent isotope fractionation during the experiments and interference-free measurement during the analytical session (Fig. S2, Online Resource).

However, as the Mg isotope composition of NIST SRM 980 may differ in individual dispatches (Galy et al. 2003), we measured Mg isotope composition of our NIST SRM 980 relative the DSM3 (Dead Sea Magnesium, Galy et al. 2003), at the Institute of Geology and Mineralogy at University of Cologne, Germany, $\left(\delta^{26}\mathrm{Mg}_{\mathrm{DSM3}}^{\mathrm{NIST980}} = +3.91 \pm 0.09‰, 2\mathrm{SD}, \mathrm{n} = 12\right)$. In the following sections, the δ^{26}Mg values of our samples are then reported relative to the DSM3 with the conversion using the equation in Young and Galy (2004):

$$\delta^{26}\mathrm{Mg} = \delta^{26}\mathrm{Mg}_{\mathrm{NIST980}}^{\mathrm{sample}} + \delta^{26}\mathrm{Mg}_{\mathrm{DSM3}}^{\mathrm{NIST980}} + 0.001 \cdot \delta^{26}\mathrm{Mg}_{\mathrm{NIST980}}^{\mathrm{sample}} \cdot \delta^{26}\mathrm{Mg}_{\mathrm{DSM3}}^{\mathrm{NIST980}} \quad (2)$$

Data calculation & Statistics

The Mg isotope compositions of aboveground shoot ($\delta^{26}\mathrm{Mg}_{\mathrm{Shoot}}$) and of the whole plant ($\delta^{26}\mathrm{Mg}_{\mathrm{Plant}}$) were calculated based on mass balance using:

$$\delta^{26}\mathrm{Mg}_{\mathrm{ShootorPlant}} = \sum\left(\delta^{26}\mathrm{Mg}_{\mathrm{i}} \times \frac{\mathrm{m_i c_i}}{\sum \mathrm{m_i c_i}}\right) \quad (3)$$

where m_i, c_i, and $\delta^{26}\mathrm{Mg_i}$ were the dry biomass, the Mg concentration, and Mg isotope composition of the plant organ *i* (root, stem, leaf, or spike), respectively. Similarly, The Mg isotope composition of the spike at the stage of maturity, where the grain and the spelt had been separated during sampling, were calculated with the data of the spelt and the grain (Table S3, Online Resource) using this mass balance equation.

The apparent difference of Mg isotope composition between different Mg pools (i.e. Mg in nutrient solution, the plant organs and the whole plant) was calculated using:

$$\Delta^{26}\mathrm{Mg}_{\mathrm{A-B}}^{=}\delta^{26}\mathrm{Mg_A} - \delta^{26}\mathrm{Mg_B} \quad (4)$$

The plant biomass, Mg concentrations and isotope compositions of the plant organs were presented as the mean values with the standard deviation (SD) of the four pot replicates. The Mg isotope composition of the

nutrient solution was given as the mean ± SD of three sample replicates. As the Mg isotope composition of the sand-released Mg was identical within error to that of the nutrient solution, we used the δ^{26}Mg value of the nutrient solution to compare with those of the plants in the following sections.

Statistical analyses were performed in SPSS Statistics (Version 22; IBM Corp.). We used a two-sample t-test to test whether the Mg concentration and isotope composition of samples in the control were significantly different from that in the low-Mg supply ($p < 0.05$). To test the significances of differences in Mg concentration and isotope composition of samples among the growth stages in the control or the low-Mg supply, the Fisher's LSD procedure and Duncan's multiple range test (DMRT) were applied ($p < 0.05$).

Results

Magnesium accumulation in plants

Magnesium concentrations of wheat plants in the control (1413 ± 49 to 2284 ± 137 mg kg^{-1}) were significantly higher than those in the treatments with low-Mg supply (451 ± 12 to 617 mg kg^{-1}) (Table S3). The mass of Mg in each plant organ was shown in Fig. 1 as calculated by multiplying the Mg concentration with the dry biomass (Table S3, Online Resource). Wheat shoots in the control and the low-Mg supply both showed continuous accumulation of Mg during plant growth. Especially after the flowering stage, the shoots accumulated significantly larger amounts of Mg than the roots (Fig. 1). With regard to each plant organ, both leaves and spikes contained significantly more Mg than roots and stems of the control (Fig. 1). At maturity, leaves and spikes in the control accumulated over 44.4% and 42.3% of the total Mg in the plants, respectively (Fig. 1). Conversely, in the low-Mg supply wheat spikes accumulated most of Mg, accounting for 73.7% of the total Mg in wheat plants at maturity (Fig. 1), while the Mg mass in the leaves showed no significant increase during plant growth (Fig. 1).

Magnesium isotope composition in plants

Preferential uptake of heavy Mg isotopes by plants was observed regardless of the amounts of the supplied Mg. Compared with the nutrient solution (δ^{26}Mg = -2.70 ± 0.06‰), wheat plants exhibited heavier Mg isotope compositions in both control ($\delta^{26}Mg_{whole\ plant}$ ranged from − 2.08 ± 0.04 to -1.28 ± 0.16‰) and low-Mg supply ($\delta^{26}Mg_{whole\ plant}$ ranged from − 1.73 to -1.47 ± 0.04‰). However, the apparent difference in Mg isotope compositions between the plant and the nutrient solution ($\Delta^{26}Mg_{plant-solution}$) varied in the two treatments. In the control, Mg isotope compositions of the wheat plants became increasingly heavier during the growth stages (Fig. 2). The enrichment of heavy Mg isotopes in plants during growth was observed to go along with the increases in δ^{26}Mg values of each individual plant organ (Fig. 3). However, in the low-Mg supply treatment, the Mg isotope composition of the wheat plants did not change as the growth proceeded, with a relatively constant $\delta^{26}Mg_{whole\ plant}$ value of about − 1.60‰. It was always 1.10‰ heavier than that in the nutrient solution (Fig. 2). The constant $\delta^{26}Mg_{whole\ plant}$ values were not reflected by the δ^{26}Mg values of each individual plant organ. The evolvement of δ^{26}Mg values during plant growth differed among plant organs (Fig. 3). Nevertheless, regardless of Mg conditions in the nutrient solution, the leaves exhibited the lightest Mg isotope compositions among all plant organs (Fig. 3). In addition, the aboveground shoots always showed lower δ^{26}Mg values compared with the roots at all growth stages, displaying a favor of lighter Mg isotopes during root-shoot transport (Table S3, Online Resource).

Discussion

Magnesium isotope fractionation by root uptake

The apparent difference in Mg isotope compositions between the whole plants and the nutrient solution ($\Delta^{26}Mg_{plant-solution}$) was associated with the Mg uptake process by the plant (Bolou-Bi et al. 2010). Even though the investigated wheat plants in the two treatments were both enriched in heavy Mg isotopes relative to the nutrient solution, the change of $\Delta^{26}Mg_{plant-solution}$ in the control differed from that in the low-Mg supply treatment (Fig. 2). The continuously increasing values of $\Delta^{26}Mg_{plant-solution}$ in the control indicated a pronounced Mg isotope fractionation due to the uptake by the rising consumption of heavy Mg isotopes from the unlimited Mg pool in the nutrient solution during plant

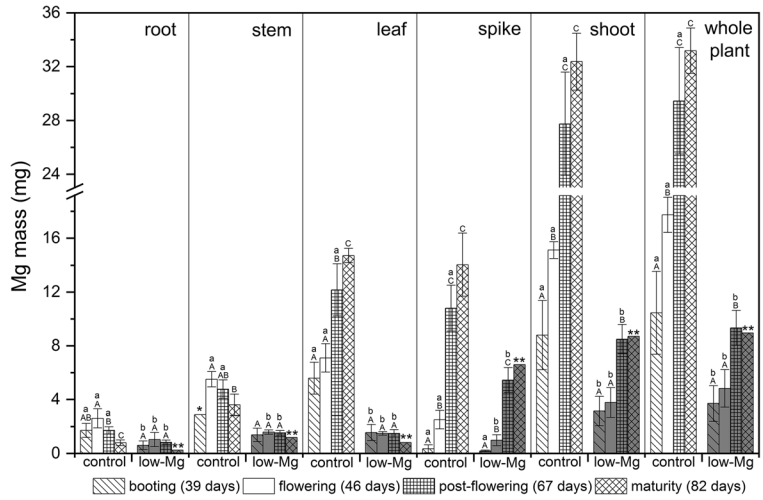

Fig. 1 Magnesium masses in each individual organs, the shoots and the whole plants during the growth stages under different Mg supplies: the control, 1 mM Mg (white); the low-Mg supply, 0.05 mM Mg (grey). Patterns in the bars represent different growth stage (from left to right: booting, flowering, post-flowering, maturity). Data are given as the mean ± SD of four experimental replicates. Different capital letters denote statistically significant differences among growth stages in each treatment, while different lowercase letters indicate statistically significant differences between two treatments at the same growth stage ($p < 0.05$). *The Mg mass of stems at the booting stage in the control is given as suggested value (Chap. 4, Online Resource). **Because there was only one data group available at maturity in low-Mg supply, statistical evaluation with regard to this data group was not conducted

growth (Fig. 2). However, the low-Mg supply resulted in a relatively constant value of $\Delta^{26}Mg_{plant-solution}$ during the plant growth (Fig. 2). As plants take up Mg via both active and passive pathways, which can fractionate Mg isotopes differently (Bolou-Bi et al. 2010), we attributed such distinct evolvements of $\Delta^{26}Mg_{plant-solution}$ to different uptake pathways adopted by the wheat plants in response to different Mg levels in the nutrient solution.

The uptake of Mg into the root cells is usually unidirectional, as revealed by isotope labelling experiments (e.g. Kuhn et al. 2000; Tanoi et al. 2014). Cross-membrane transport of Mg located at the root level is then the main process that induces Mg isotope fractionation between plants and the growth media as found by Bolou-Bi et al. (2010) in cultivated clover and rye grass with pot experiments. Magnesium can be taken up by plant roots either via Mg-specific transporters (Gebert et al. 2009; Li et al. 2001) or via non-selective cation channels or the K/Ca-channels in the plasma membrane (Hermans et al. 2013; Tang and Luan 2017). The former is an active transport pathway which is energy-consumed and requires Mg to be bound to membrane proteins before entering the root cells, whereas the latter is a passive pathway in which Mg migrates mainly as free cations (Marschner 2011). The uptake through the two pathways may result in different Mg isotope compositions of the whole plant, as binding with organic molecules is known to induce an enrichment of heavy Mg isotopes in Mg-organic complexes, as proven by previous studies based on both laboratory experiments and theoretical calculations (e.g. Black et al. 2007; Bolou-Bi et al. 2010; Moynier and Fujii 2017; Pokharel et al. 2018). By contrast, cross-membrane transport of free Mg cations may only lead to limited isotope fractionation and thus limited difference in $\delta^{26}Mg$ values between the whole plants and the growth media (Pokharel et al. 2017, 2018). Since a plant may utilize both pathways based on the vegetal demand for Mg and on the Mg availability in the growth media, the degree of Mg isotope fractionation induced by root uptake is thus further dependent on the participation of these two pathways as already observed in rice (Cai et al. 2012; Tanoi et al. 2014). In the present study, the wheat plants in the control exhibited a Mg isotope composition close to the nutrient solution at the booting stage (Fig. 2). This indicates that the passive transport pathway was likely the dominant one. When plants grew older, their Mg demands consequently increased, and the active transport pathway played an increasing role in Mg uptake. The increasing involvement of the active transport pathway thus caused the $\delta^{26}Mg_{whole\ plant}$ values to become

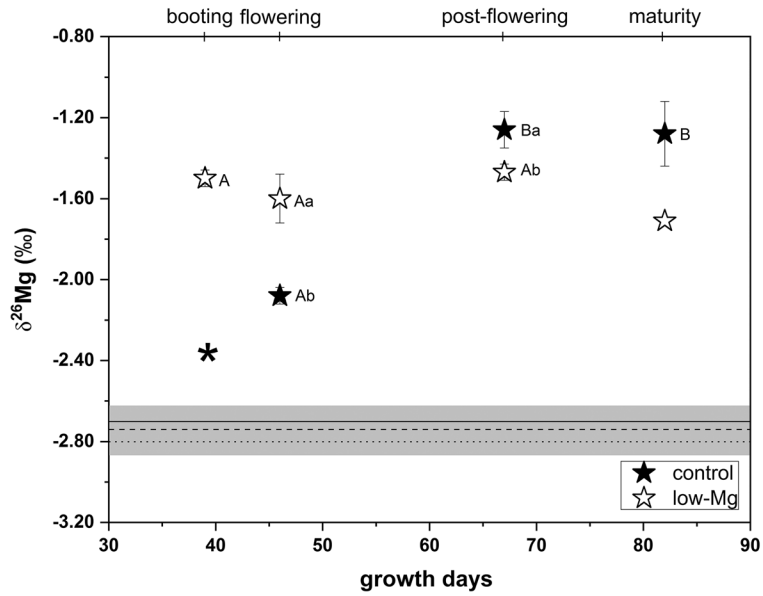

Fig. 2 Changes of Mg isotope compositions of the whole plants during the growth stages under different Mg supplies. Data are calculated based on mass balance equation (Eq. 3) and given as the mean ± SD of four experimental replicates. Different capital letters denote statistically significant differences among growth stages in each treatment, while different lowercase letters indicate statistically significant differences between two treatments at the same growth stage ($p < 0.05$). The Mg isotope compositions of the nutrient solution (solid line, $\delta^{26}Mg_{nutrient\ solution}$ = -2.70 ± 0.06‰) and of the exchangeable Mg pool in the quartz sand (dotted line, $\delta^{26}Mg_{sand-released}$ = -2.81 ± 0.11‰) are identical within error. The dashed line and the grey area (solid line, $\delta^{26}Mg$ = -2.74 ± 0.13‰) represent the $\delta^{26}Mg$ of the mixed pool of $Mg_{sand-released}$ and $Mg_{nutrient\ solution}$ in the low-Mg supply (Chap. 2, Online Resource). *Estimated value of the $\delta^{26}Mg_{whole\ plant}$ at the booting stage in the control (Chap. 4, Online Resource)

heavier during plant growth (Fig. 2). On the contrary, in the low-Mg supply, wheat plants displayed heavy Mg isotope compositions already at the booting stage (Fig. 2), reflecting that the active pathway was dominant in Mg uptake. Moreover, there was limited variation of $\Delta^{26}Mg_{plant-solution}$ during plant growth. It indicated the active pathway to be the main strategy for Mg uptake along the life cycle of the wheat plants when Mg was supplied insufficiently.

Our data are in line with Tanoi et al. (2014) who reported that Mg uptake through active pathway was enhanced by Mg deficiency. They explained this finding by the fact that the plant requires Mg to sustain essential physiological functions even if this occurs at the expense of energy when Mg supply is limited. In addition, our results support the hypothesis that plants can modulate the uptake pathways by changing the participations of the passive and active pathways when the nutrient concentration varies (Epstein and Bloom 2005). However, it remains unclear that at which Mg concentration plants start to adjust the uptake pathways. Nevertheless, our data provide clear evidence that the Mg isotope composition of a plant, as well as its changes during plant growth, can directly reflect the uptake pathways, and thus indicate for the degree that whether cross-membrane transport is dominated by active, carrier-mediated transport mechanisms or by passive Mg-permeable channels. Directly correlating the extent of Mg isotope fractionation to the monitoring of the different transport pathways, however, warrants further investigation.

Speciation calculation with Visual MINTEQ v3.0 showed that in the nutrient solution, free Mg cations accounted for 96.5% and 99.7% of the total Mg content in the control and the low-Mg supply treatments, respectively. Hence, differences in the Mg speciation due to distinct Mg concentrations in nutrient solution were negligibly small if at all present between the two treatments. It is worth noting that the low-Mg supply may enhance the root exudation of organic acids with low molecular masses (Ohta and Hiura 2016). Such exudation processes alter the Mg availability in rhizosphere (Marschner et al. 1987). However, in the present study the exact amount of Mg from the sands and its Mg speciation utilized by plants was unknown. As both free Mg cations and Mg-organic acid complexes can both be

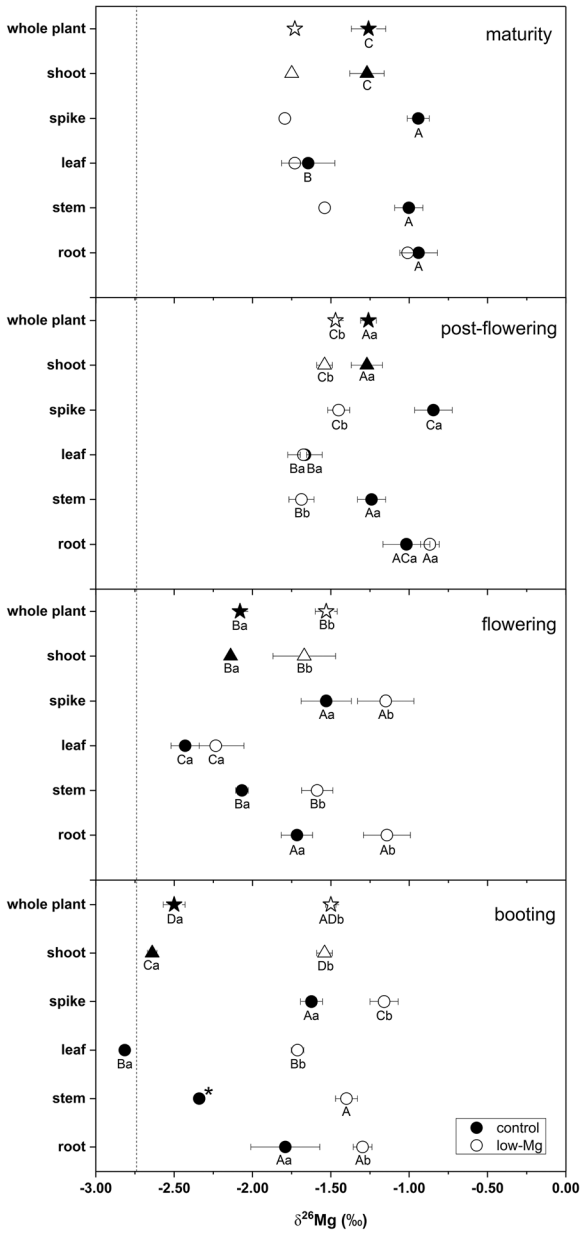

Fig. 3 Magnesium isotope compositions of plant organs, and of the shoots and the whole plants during the growth stages in the control (1 mM Mg) and the low-Mg supply (0.05 mM Mg). Data are given as the mean ± SD of four experimental replicates. The vertical dashed line is the mean δ^{26}Mg value (-2.74 ± 0.13‰) of the mixed pool of $Mg_{sand-released}$ and $Mg_{nutrient\ solution}$ (Chap. 2, Online Resource). Different capital letters at each growth stage denote statistically significant differences among plant organs in each treatment, while different lowercase letters at each growth stage indicate statistically significant differences of the same plant organs between two treatments ($p < 0.05$). *Estimation of the δ^{26}Mg of the stem at the booting stage in the control is given in the Chap. 4, Online Resource

taken up by roots, it seemed reasonable to assume that the Mg isotope composition of rhizospheric Mg-organic acid complexes varied within the pool of exchangeable Mg in the quartz sand (Chap. 2, Online Resource), and the root exudation would not significantly affect the root uptake-induced Mg isotope fractionation.

Magnesium isotope fractionation by root-shoot translocation

After entering in root cells, Mg can be loaded into the xylem rapidly and transported upwards with the transpiration stream to upper plant organs of active transpiration and photosynthesis, such as expanded leaves. Our results showed that such root-shoot translocation process fractionated Mg isotopes, leading to an enrichment of isotopically light Mg in the shoots relative to the roots in both treatments (Fig. 3). The positive values of $\Delta^{26}Mg_{root-shoot}$ (Fig. 4), ranging from + 0.26 ± 0.06 to + 0.85 ± 0.18‰ for the control and from + 0.24 ± 0.08 to + 0.74 ± 0.00‰ for the low-Mg supply, respectively, corresponded on the same order of magnitude to the results of previous studies on rye grass (Bolou-Bi et al. 2010) and rice (Gao et al. 2018). However, changes of $\Delta^{26}Mg_{root-shoot}$ with plant growth in the low-Mg supply differed from those in the control (Fig. 4). The values of $\Delta^{26}Mg_{root-shoot}$ in the low-Mg supply increased by 0.5‰ from booting to maturity, while those in the control decreased with plant growth. Such contrary evolvements of $\Delta^{26}Mg_{root-shoot}$ indicated that low-Mg supply did not only affect the root uptake of Mg but also the upwards translocation of Mg from root to shoot. As Mg is transferred from roots to shoots through xylem loading, the apparent difference of Mg isotope compositions between roots and shoots was thus likely resulted from the xylem loading process. However, the exact mechanism answering for xylem Mg loading remains unclear (Chen et al. 2018). To date, several transport systems are suggested to regulate Mg entering into the xylem vessels (Hermans et al. 2013). First, MRS/MGT-family protein (implicating AtMRS2-2) is involved in xylem loading process in rice roots as verified by Tanoi et al. (2011). Second, one of the cyclic nucleotide-gated channels, CNGC10 in mature *Arabidopsis* plants, has also been reported to be capable of transporting Mg cations into the xylem (Li et al. 2005; Guo et al. 2010). Finally, the *Mg/H*$^+$ *Exchanger 1* localized in the vacuolar membrane may control the amount of Mg that stored in the cytosol of parenchyma cells for xylem

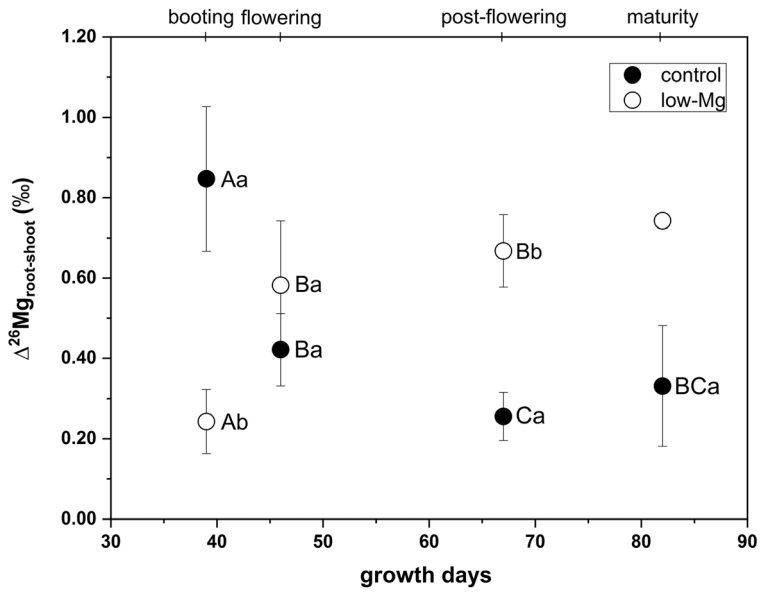

Fig. 4 Apparent differences of Mg isotope compositions between the roots and the shoots ($\Delta^{26}Mg_{root-shoot}$) in the control (1 mM Mg, solid circles) and in the low-Mg supply (0.05 mM Mg, open circles) during plant growth. Data are given as the mean ± SD of four experimental replicates. Different capital letters denote statistically significant differences among growth stages in each treatment, while different lowercase letters indicate statistically significant differences between two treatments at the same growth stage ($p < 0.05$)

loading (Shaul et al. 1999; Kobayashi and Tanoi 2015). It can be inferred from our data that similar to the root uptake of Mg, these systems presumably also have different preference of light or heavy Mg isotopes. In this case, the Mg isotope fractionation induced by xylem loading process is thus determined by the Mg speciation (Mg-organic complexes or free cations) during membrane transport. If Mg is first bound to membrane proteins and then transported as Mg-organic complexes, the Mg pool in the xylem is presumed to become isotopically heavier. On the contrary, direct transport of free Mg cations likely induces limited variation or a negative shift of the δ^{26}Mg values in the xylem. In addition, the participation of each pathway in xylem loading may vary depending on the level of supplied Mg for plant growth that can also change the Mg isotope composition in xylem sap. We thus envision that specific measurements of Mg isotope compositions in e.g. the xylem sap and the cytosol of xylem parenchyma cells could help clarify the xylem loading process of Mg under different Mg conditions.

Magnesium isotope fractionation during grain filling by remobilization

During grain filling, the wheat spikes in both the control and the low-Mg supply displayed a remarkable accumulation of more Mg than at the booting growth stage (Fig. 1), indicating a higher demand for nutrients in the productive organs at reproductive growth stage. However, accumulation of Mg in wheat leaves at the booting stage was only observed in the control (Fig. 1). Low-Mg supply resulted in a limited variation of Mg mass in leaves (Fig. 1). By plotting the δ^{26}Mg value as a function of Mg mass (Fig. 5), the Mg isotopes in the wheat spikes and leaves of the two treatments fractionated conversely with the plant growth. In the control, the wheat spikes showed an enrichment of heavy Mg isotopes, whereas the wheat spikes in the low-Mg supply became isotopically lighter with increasingly accumulation of Mg (Fig. 5a). Wheat leaves in the control displayed similar trend to the spikes, while that in the low-Mg supply was much different (Fig. 5b). Such distinct patterns of Mg isotope fractionation in wheat spikes and leaves in the two treatments indicated that the Mg source for grain filling differed, possibly due to the variation of Mg remobilization processes within plants under different Mg supplies. Reproductive organs such as grains typically exhibit high nutritional demand but a low transpiration rate. Hence, the spikes are expected to be mainly supplied with Mg through phloem transport instead of xylem transport (Hermans et al. 2013; Wiggenhauser et al. 2018; Pearson et al. 1995). The Mg isotope composition of the phloem therefore directly affects that of the reproductive organs. As phloem-mobile element, Mg can be remobilized from older leaves to younger leaves or reproductive organs after chlorophyll decomposition during leaf senescence (Langmeier et al. 1993, Nooden et al. 1997). A recent study by Peng et al. (2019) showed that limited Mg supply accelerated the process of chlorophyll breakdown

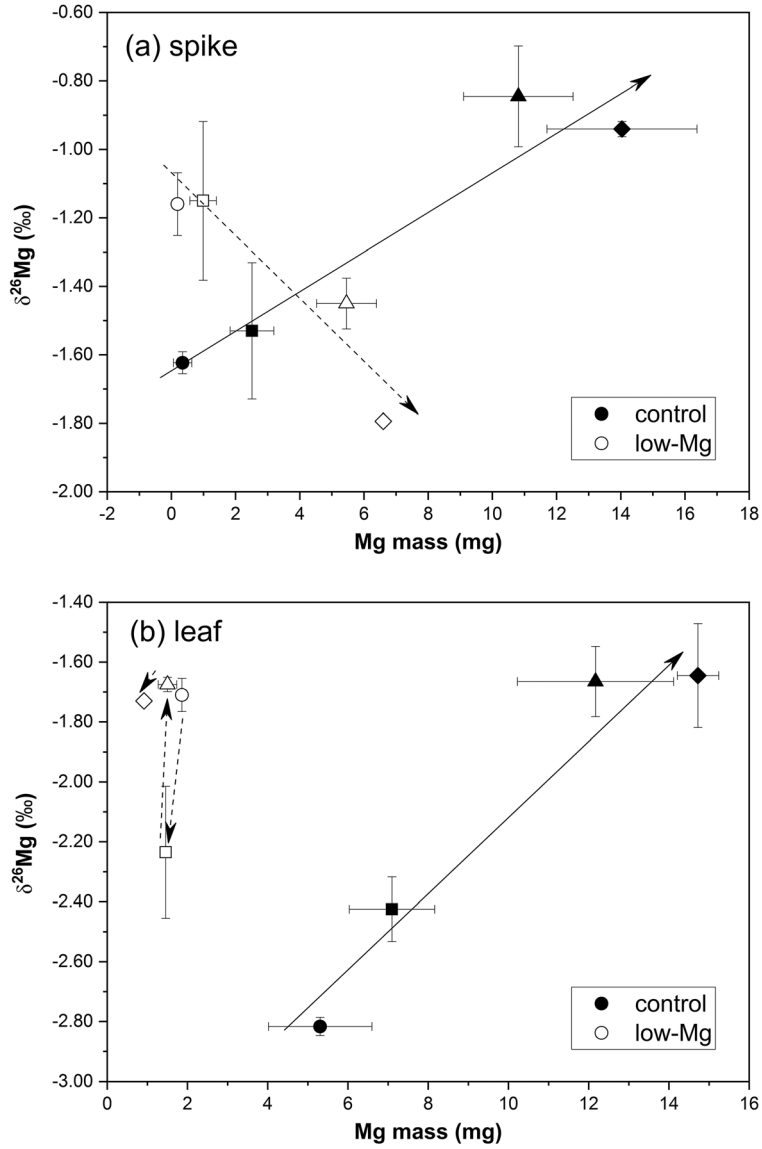

Fig. 5 Relationships between Mg isotope compositions and Mg masses of the wheat spikes (a) and leaves (b) in the control (1 mM Mg, solid symbols) and the low-Mg supply (0.05 mM Mg, open symbols), respectively. The growth cycle of the plants is marked with arrows. Different symbols represent the growth stages of booting (circle), flowering (square), post-flowering (triangle), and maturity (diamond), respectively. Data are given as the mean ± SD of four experimental replicates

and subsequent release of Mg even from mid-aged leaves for Mg remobilization in rice. This suggests that, in our study, the remobilization of Mg was presumably already advanced at the flowering stage in the low-Mg supply, which induced a marked outflux of Mg from the leaves in this period. Compared with other plant organs, isotopically light Mg, mainly located in chlorophylls, is usually enriched in the leaves (Galy et al. 2001; Young and Galy 2004; Black et al. 2006, 2008; Ra and Kitagawa 2007; Ra et al. 2010), which results in the leaves being isotopically the lightest among plant organs. Consequently, a breakdown of chlorophyll molecules can release isotopically light Mg into the phloem, leading to the phloem sap becoming enriched in light Mg isotopes. Due to the depletion of isotopically light Mg, the δ^{26}Mg value of Mg source (leaf) increased after flowering in the low-Mg supply, while the Mg sink (spike) became isotopically lighter as it was fed by the isotopically light Mg via the phloem transport (Fig. 3). In this regard our results agree with the study of Kimmig et al. (2018) who showed that yellow leaves of sugar maple exhibited higher δ^{26}Mg values than the green ones as light Mg isotopes were preferentially remobilized from the Mg sources (already yellowed leaves). As wheat plants also take up additional Mg from the growth media during grain filling stage, it is difficult to quantify the in- and outflux of Mg in leaves.

Unlike the control, the net-remobilization of Mg in leaves of the low-Mg supply seemed to be balanced after flowering, as suggested by the limited variation of Mg mass in leaves (Fig. 1). Therefore, the discrepancy in Mg isotope composition of wheat leaves and spikes indicated that different mechanisms were involved in the Mg remobilization processes under the two different Mg supplies.

Overall, our findings supported the hypothesis that Mg deficiency could enhance the remobilization processes of Mg from the source to sink organs through the phloem transport (Billard et al. 2016; Peng et al. 2019). When Mg is low in the growth media, the chlorophylls in leaves are expected to break down and release Mg to move towards the wheat spikes with the phloem sap. In this regard, analyzing the Mg isotope composition of each plant organ at different growth stages provides a novel and integrative tool to identify different Mg utilization patterns of the plants, which may also be used to study Mg acquisition and translocation strategies under field conditions without sophisticated transporter analyses. Noticeably, recent studies on Zn and Cd transport within rice showed that apart from the phloem transport of stored Zn and Cd from the leaves, xylem-to-phloem transfer at the stem nodes also played a role in mobilizing Zn and Cd to the grains via the phloem. This indicates that Zn and Cd could be loaded into the phloem and then directly transported into the grains without leaf storage (Nishiyama et al. 2013; Yoneyama et al. 2010, 2015). The direct influx of the elements from the xylem would then result in the isotope composition of the grains being different from that receiving the element remobilized from the leaves via phloem. Whether this kind of transport is also valid for Mg in wheat and its effect on Mg isotope compositions in the wheat spikes/grains warrants further studies.

Implications

Nowadays, Mg deficiency in plants occurs increasingly both in agriculture and forestry at a worldwide scale (Marschner 2011). However, plants can develop various strategies to deal with frequently encountered environmental stress like nutrient deficiencies (Cai et al. 2012). Our work traced the Mg isotope compositions of the widely grown agronomic crop wheat across its life cycle under both control and low-Mg supply conditions. Variations of the Mg isotope compositions in different plant organs demonstrated that different mechanisms were potentially involved in the plant's response to nutrient deficiency. The uptake of Mg was expected to be controlled by both active and passive pathways in the wheat root, with the active pathway dominating under low-Mg conditions throughout the life cycle of wheat plants. On the contrary, the wheat plants supplied with sufficient Mg altered the participations of these two pathways during plant growth depending on its Mg demand. In addition, low-Mg supply is expected to enhance the break-down of chlorophylls in the leaves, as well as the remobilization process of the released Mg from the leaves to spikes. To date, our knowledge about the contribution of different processes involved in the Mg uptake strategy, as well as the long-distance transport of Mg within plant is still limited. Our study showed that the evolvements of Mg isotope compositions of whole wheat plants and individual plant organs during growth reflected the utilization of distinct uptake pathways and distinct Mg remobilization processes within the plants, respectively. Studying stable isotopes combined with mass balance calculation can thus be a useful tool to assist the understanding of the molecular biology of phytophysiological activities, e.g., on how plants survive through strategic adjustment at different growth stages under limited nutrient supply, and to which extent different biological processes contribute to the plant growth.

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