VIEWPOINT ARTICLE

Linear relationships between shoot magnesium and calcium concentrations among angiosperm species are associated with cell wall chemistry

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Running Title: Cell wall chemistry explains correlations of shoot Mg and Ca concentrations

ABSTRACT

- **Background** Linear relationships are commonly observed between shoot magnesium ($[Mg]_{shoot}$) and shoot calcium ($[Ca]_{shoot}$) concentrations among angiosperm species growing in the same environment.
- **Scope and conclusions** This article argues that, in plants that do not exhibit “luxury” accumulation of Mg or Ca, (1) distinct stoichiometric relationships between $[Mg]_{shoot}$ and $[Ca]_{shoot}$ are exhibited by at least three groups of angiosperm species, namely commelinid monocots, eudicots excluding Caryophyllales, and Caryophyllales species, (2) that these relationships are determined by cell wall chemistry and the Mg/Ca mass quotients in their cell walls, (3) that differences between species in $[Mg]_{shoot}$ and $[Ca]_{shoot}$ within each group are associated with differences in the cation exchange capacity (CEC) of the cell walls of different species, and (4) that Caryophyllales constitutively accumulate more Mg in their vacuoles than other angiosperm species when grown without a supra-sufficient Mg supply.

Key words: Angiosperm, Calcium (Ca), Cation exchange capacity (CEC), Caryophyllales, Cell wall, Commelinid monocot, Magnesium (Mg), Poales, Shoot, Stoichiometry, Vacuole.

INTRODUCTION

Calcium (Ca) and magnesium (Mg) are both plant nutrients (Hawkesford *et al.*, 2012). Calcium is essential for cell wall and membrane integrity and for cytosolic signalling. Magnesium is required for protein synthesis, energy metabolism, and photosynthesis as a constituent of chlorophyll. Although each of these elements has unique biological functions, linear relationships between shoot Ca concentration ($[\mathrm{Ca}]_{\mathrm{shoot}}$) and shoot Mg concentration ($[\mathrm{Mg}]_{\mathrm{shoot}}$) are commonly observed among angiosperm species growing in the same environment, with the exception of species of the Caryophyllales order (White *et al.*, 2015). These relationships have been observed in both field (Garten, 1976; Thompson *et al.*, 1997; Watanabe *et al.*, 2007; Fyllas *et al.*, 2009; White *et al.*, 2012) and glasshouse studies (Broadley *et al.*, 2004; White *et al.*, 2015). This article suggests an anatomical basis for such relationships.

SHOOT CALCIUM AND MAGNESIUM CONCENTRATIONS CORRELATE WITH CELL WALL CATION EXCHANGE CAPACITY

The $[\mathrm{Ca}]_{\mathrm{shoot}}$ of Ca-replete angiosperms generally lies between 1 and 50 mg Ca g^{-1} dry matter (DM) and the $[\mathrm{Mg}]_{\mathrm{shoot}}$ of Mg-replete angiosperms between 1 and 10 mg Mg g^{-1} DM, depending on plant species and growth conditions (Hawkesford *et al.*, 2012). Eudicot species, and species of the non-commelinid monocots, generally have greater $[\mathrm{Ca}]_{\mathrm{shoot}}$ and $[\mathrm{Mg}]_{\mathrm{shoot}}$ than species of the commelinid monocots (**Figure 1a,b**; Thompson *et al.*, 1997; Broadley *et al.*, 2003, 2004; Watanabe *et al.*, 2007; White *et al.*, 2012, 2015). There are often strong correlations in both relative $[\mathrm{Ca}]_{\mathrm{shoot}}$ and relative $[\mathrm{Mg}]_{\mathrm{shoot}}$ of angiosperm species between studies, indicating that the ranking of angiosperm species for both $[Ca]_{shoot}$ and $[Mg]_{shoot}$ is largely independent of environment (Broadley *et al.*, 2003; White *et al.*, 2012).

Asher and Ozanne (1967) observed that $[Ca]_{shoot}$ was directly related to the cation exchange capacity (CEC) of root cell walls among angiosperm species and this has been confirmed in other studies (White and Broadley, 2003; Ray and George, 2011). It is observed that the gradient of the relationship between $[Ca]_{shoot}$ and root CEC is similar for all angiosperm species (**Figure 1c**). The CEC of cell walls in the shoot is generally similar to, or greater than, that of root cell walls (Knight *et al.*, 1973). When the same tissue is assayed in plants grown under similar conditions, cell wall CEC is generally greater in eudicots and non-commelinid monocots than in commelinid monocots (**Figure 1c,d**; White and Broadley, 2003). Estimates of the CEC of root cell walls range from 45 - 1019 meq kg^{-1} DM in eudicots, 180 - 389 meq kg^{-1} DM in non-commelinid monocots, and 10 - 578 meq kg^{-1} DM in commelinid monocots (White and Broadley, 2003). An equivalent is the number of moles of an ion multiplied by the valence of that ion (e.g. 1 meq = 0.5 mmol for Ca^{2+} and Mg^{2+}). Cell wall CEC is not constant for a plant species but can vary with development, growth conditions and the tissue sampled (Heintze, 1961). The CEC is dominated by the free carboxyl groups of polygalacturonic acids (pectins) in the middle lamella of cell walls (White and Broadley, 2003; Taiz *et al.*, 2015). Although there are many different pectin structures (Ridley *et al.*, 2001; Sénéchal *et al.*, 2014; Dahler and Braybrook, 2015; Park and Cosgrove, 2015; Anderson, 2016; Bidhandi and Geitmann, 2016) and pectin content can differ between and within tissues, change with development, and respond to both abiotic and biotic challenges (Popper *et al.*, 2011; Sénéchal *et al.*, 2014; Dahler and Braybrook, 2015; Le Gall *et al.*, 2015; Park and Cosgrove, 2015; Anderson, 2016), the cell walls of eudicots and non-commelinid monocots generally have similar pectin contents, and both have more pectin than cell walls of commelinid monocots (Figure 2; Jarvis *et al.*, 1988; Harris *et al.*, 1997; Smith and Harris, 1999; Popper *et al.*, 2011; Banasiak, 2015). The cell walls of eudicots excluding Caryophyllales, Caryophyllales, non-commelinid monocots and commelinid monocots also differ in other cell wall properties (Figure 2; Harris and Tretheway, 2010; Popper *et al.*, 2011; Banasiak, 2015). In particular, non-commelind monocots generally contain greater amounts of xyloglucans, mixed linkage glucans and ester-related p-coumaric acids than eudicots and non-commelinid monocots (Banasiak, 2015; Hatfield *et al.*, 2017) and the hemicelluloses of non-commelinid monocots and the rhamnogalacturonan-1 pectins of Caryophyllales can be covalently crosslinked by feruoylation (Ridley *et al.*, 2001; Harris and Tretheway, 2010; Hatfield *et al.*, 2017). It has been speculated that the CEC of cell walls might influence free Ca^{2+} concentrations in the apoplast and, thereby, cell signalling (Hepler and Winship, 2010), but there is, as yet, no direct evidence that cell wall CEC affects Ca^{2+} signalling across the plasma membrane of mature plant cells.

Cell walls also bind substantial amounts of Mg (Hawkesford *et al.*, 2012) and $[Mg]_{shoot}$, like $[Ca]_{shoot}$, is correlated with cell wall CEC among angiosperm species (**Figure 1d**). However, the gradient of the relationship between $[Mg]_{shoot}$ and root CEC is greater among commelinid monocots than among eudicots, with the exception of Caryophyllales species (**Figure 1d**). Since the relationship between $[Ca]_{shoot}$ and root CEC does not differ between angiosperm species, this data indicates that cell walls of commelinid monocots have a lower Ca/Mg selectivity than cell walls of most eudicots.

ESTIMATES OF CALCIUM AND MAGNESIUM CONCENTRATIONS IN CELL WALLS AND INTRACELLULAR COMPARTMENTS OF SHOOTS

Estimates of the Ca concentration in cell walls of eudicot shoots range from 0.47 to 38.9 mg Ca g^{-1} DM (Nakajima *et al.*, 1981; Goldberg *et al.*, 1986; Mühling and Sattelmacher, 1995; Miklós *et al.*, 2000, Carr *et al.*, 2003; Liu *et al.*, 2015), which convert to values of 11.7 to 970 mM if it is assumed that water makes up about two thirds of cell wall mass in growing tissues (Cosgrove, 1997), and Ca concentration in cell walls of commelinid monocot shoots range from 0.26 to 3.0 mg Ca g^{-1} DM, which equates to 6.5 to 74.8 mM (Turan *et al.*, 2009; Zeng *et al.*, 2010). Estimates of the Mg concentration in cell walls of eudicot shoots range from 0.024 to 0.99 mg Mg g^{-1} DM (Nakajima *et al.*, 1981; Mühling and Sattelmacher, 1995; Carr *et al.*, 2003) and the Mg concentration in rice shoots has been estimated to be 0.072 mg Mg g^{-1} DM (Zeng *et al.*, 2010), which are equivalent to 0.99 to 40.7 mM and 2.96 mM, respectively. Estimates of Ca/Mg mass quotients in shoot cell walls of eudicots range from 5.63 to 17.60 (Nakajima *et al.*, 1981; Mühling and Sattelmacher, 1995; Carr *et al.*, 2003) and that of rice shoots has been estimated to be 3.56 (Zeng *et al.*, 2010). Cell walls of eudicot shoots generally contain between 70-99% of the total tissue Ca, although values as low as 17% have been reported (Mühling and Sattelmacher, 1995), but only 1 to 11% of the total tissue Mg (Nakajima *et al.*, 1981; Mühling and Sattelmacher, 1995; Miklós *et al.*, 2000; Liu *et al.*, 2015), although 80% of the Mg in the first trifoliate leaf of a subterranean clover plant was found to be associated with a fibre fraction by Scott and Robson (1990). Greater concentrations of Ca and Mg, and greater Ca/Mg mass quotients, have also been observed in root cell walls of eudicots than in those of commelinid monocots (e.g. Mehlich, 1953) and the Ca/Mg selectivity of root cell walls has been found to increase with increasing CEC among Poales species (Waquant, 1977).

In plants that contain no precipitated Ca salts, the Ca concentration in shoot vacuoles generally lies between 2 and 20 mM, but can reach 80 mM in some cells, and that in chloroplasts is between 7 and 12 mM (Carr *et al.*, 2003; Stael *et al.*, 2012). The open cytoplasm contains between 0.1 and 1 mM Ca and the endoplasmic reticulum (ER), mitochondria and nuclei contain about 2 mM Ca (White and Broadley, 2003; Stael *et al.*, 2012). Chloroplasts contain 5 to 10% of total leaf Mg in Mg-replete plants, but up to 20 to 35% of leaf Mg in Mg-deficient plants (Scott and Robson, 1990; Hawkesford *et al.*, 2012). The Mg is present in chlorophyll, at a concentration of about 100 mM, and in solution, at a concentration of 5 to 20 mM (Shaul, 2002). Between 60 and 90% of the total Mg in leaves of Mg-replete plants is in a water-soluble form (Hawkesford *et al.*, 2012). In Mg-replete plants, vacuolar Mg concentrations generally lie between 3 and 20 mM, but Mg concentrations of up to 120 mM have been reported in some cells (Shaul, 2002; Carr *et al.*, 2003; Hawkesford *et al.*, 2012). It is thought that the open cytosol contains 2 to 10 mM Mg, mitochondria contain 7 - 11 mM Mg, and the ER and nuclei contain 10 - 20 mM Mg (Hawkesford *et al.*, 2012; Gout *et al.*, 2014). These values are similar to the Mg concentrations found in these organelles in animal cells (Romani, 2011). Cameron *et al.* (1984) estimated that the open cytoplasm of onion root cells contained 16-32 mmol Ca kg^{-1} DM and 67-156 mmol Mg kg^{-1} DM (Ca/Mg mass quotient = 0.34-0.39) and nuclei contained 9-36 mmol Ca kg^{-1} DM and 61-139 mmol Mg kg^{-1} DM (Ca/Mg mass quotient = 0.24-0.43).

THE CONTRIBUTIONS OF CELL WALLS AND INTRACELLULAR COMPARTMENTS TO SHOOT CALCIUM AND MAGNESIUM CONCENTRATIONS

Although it is acknowledged that both leaves, and cell types within leaves, differ in their Ca and Mg concentrations (Conn and Gilliham, 2010; Hawkesford *et al.*, 2012), this article refers to a composite shoot that integrates these differences. This composite shoot is considered to have several features with different Ca and Mg concentrations and distinct

Ca/Mg mass quotients: the cell wall, the cytoplasm, comprising the open cytosol, endoplasmic reticulum, nucleus, mitochondria and chloroplasts, and the vacuole. Their approximate contributions to the volume of a mature leaf are assumed to be: cell wall 6%, open cytoplasm 5%, chloroplasts 5%, nuclei 0.5%, mitochondria 1%, ER 0.5% and vacuole 82% (**Table 1**; Heldt and Piechulla 2010; Hawkesford *et al.*, 2012). However, it must be stressed that these contributions can vary greatly between plant species, in leaves of different ages, and in plant grown under contrasting environmental conditions.

Broadley *et al.* (2004) suggested that there was a linear relationship between $[Mg]_{shoot}$ and $[Ca]_{shoot}$ among all angiosperm species, with the exception of Caryophyllales that had greater $[Mg]_{shoot}$ at any given $[Ca]_{shoot}$ than other angiosperm species. They also suggested that the commelinid monocots had smaller $[Mg]_{shoot}$ and $[Ca]_{shoot}$ than other angiosperm species. In the dataset assembled here, which includes more species than the Broadley *et al.* (2004) study, it appears that the data can be separated into at least three groups: (1) commelinid monocots (Arecales, Commelinales, Poales, Zingiberales), (2) eudicots excluding Caryophyllales, and (3) Caryophylalles species (**Figure 1a,b**). The non-commelinid monocots might form a fourth group. In all groups there appears to be a linear relationship between $[Mg]_{shoot}$ and $[Ca]_{shoot}$, but the gradient of this relationship and $[Mg]_{shoot}$ at zero $[Ca]_{shoot}$ differ between groups (**Figure 1a,b**). The equations for linear regressions of the relationships between $[Mg]_{shoot}$ and $[Ca]_{shoot}$, both expressed as mg g^{-1} DM, are $[Mg]_{shoot} = 1.09 \pm 0.20 + (0.25 \pm 0.02 \text{ x } [Ca]_{shoot})$ for commelinid monocots, $[Mg]_{shoot} = 2.17 \pm 0.21 + (0.10 \pm 0.01 \text{ x } [Ca]_{shoot})$ for eudicots excluding Caryophyllales, and $[Mg]_{shoot} = 4.68 \pm 0.92 + (0.17 \pm 0.08 \text{ x } [Ca]_{shoot})$ for Caryophyllales species. The commelinid monocots have a greater gradient and smaller $[Mg]_{shoot}$ at zero $[Ca]_{shoot}$ than the eudicots, whilst the Caryophyllales have an intermediate gradient but a considerably greater $[Mg]_{shoot}$ at zero $[Ca]_{shoot}$ than other angiosperm species. In agreement with Broadley *et al.* (2004), the magnitude of $[\mathrm{Mg}]_{\mathrm{shoot}}$ and $[\mathrm{Ca}]_{\mathrm{shoot}}$ are generally less in the commelinid monocots than other angiosperm species.

The dataset presented in Figure 1 comprises data from six experiments undertaken in hydroponics using the same nutrient solution (White *et al.*, 2017; Neugebauer *et al.*, 2018). Although experiments in hydroponics might underestimate the consequences of vagaries in the phytoavailability of nutrients and toxic elements in soil and the intimate interactions between the root and the soil on the shoot ionome (Brown *et al.*, 2017; Neugebauer *et al.*, 2018), it is noteworthy that the gradients of the relationships between $[\mathrm{Mg}]_{\mathrm{shoot}}$ and $[\mathrm{Ca}]_{\mathrm{shoot}}$ among angiosperm species obtained in the hydroponic system described by White *et al.* (2017) are similar to those obtained in more natural environments (Broadley *et al.*, 2004; White *et al.*, 2012).

If the relationship between $[\mathrm{Mg}]_{\mathrm{shoot}}$ and $[\mathrm{Ca}]_{\mathrm{shoot}}$ can be attributed to variation in a single, common anatomical feature within each group, then the reciprocal of the gradient of this relationship is the Ca/Mg mass quotient of that anatomical feature. In the dataset compiled here, this is 4.00 for commelinid monocots, 10.0 for eudicots excluding the Caryophyllales, and 5.88 for Caryophyllales species. For comparison, the equivalent values for all angiosperms excluding Caryophyllales in the hydroponic study of Broadley *et al.* (2003) and the field studies of Garten *et al.* (1976) and Thompson *et al.* (1997) were all 7.7, for the field study of White *et al.* (2012) it was 8.9, and for the hydroponic study of White *et al.* (2015) it was 11.1. These values correspond to the Ca/Mg mass quotients reported for cell walls of rice shoots (3.56; Zeng *et al.*, 2010) and eudicots (5.63-17.60; Nakajima *et al.*, 1981; Mühling and Sattelmacher 1995; Carr *et al.*, 2003). It is, therefore, possible that the stoichiometric relationships between $[\mathrm{Mg}]_{\mathrm{shoot}}$ and $[\mathrm{Ca}]_{\mathrm{shoot}}$ of groups of angiosperm species reflects the relative binding of these cations in their cell walls, which differs between the three groups. The absolute binding capacity of cell walls of individual species within these groups is likely to be related their characteristic cell wall CEC.

If the plants growing hydroponically in the dataset reported here do not exhibit "luxury" accumulation of either Ca or Mg, the minimal intracellular requirement for Ca and Mg for cellular functions might be estimated from the minimal concentrations of these elements in cellular compartments reported in plants growing with adequate nutrition (**Table 1**). Expressed on a leaf volume basis the minimal intracellular concentrations are 2.04 mM Ca and 7.98 mM Mg, which equate to 816 mg Ca kg^{-1} leaf DM and 1,939 mg Mg kg^{-1} leaf DM assuming a leaf DM/FW quotient of 0.10 (Broadley *et al.*, 2003). For comparison, simple linear regressions of the data for $[Mg]_{shoot}$ and $[Ca]_{shoot}$ suggest $[Mg]_{shoot}$ of 1,294 and 2,252 mg kg^{-1} leaf DM for commelinid monocots and eudicots excluding Caryophyllales at a $[Ca]_{shoot}$ of 816 mg Ca kg^{-1} leaf DM, respectively. Assuming minimal leaf concentrations of 1 mg Ca g^{-1} DM and 2 mg Mg g^{-1} DM for a commelinid monocot, cell wall concentrations of 0.37 mg Ca g^{-1} cell wall DM and 0.12 mg Mg g^{-1} cell wall DM can be calculated based on a the cell wall contributing 50% of the total leaf dry matter (Sugiyama and Shimazaki, 2007). Both these values are similar to estimates of the Ca concentration (0.26-3.0 mg Ca g^{-1} DM; Turan *et al.*, 2009; Zeng *et al.*, 2010) and Mg concentration (0.072 mg Mg g^{-1} DM; Zeng *et al.*, 2010) in cell walls of monocot leaves, and the calculated cell wall Ca/Mg mass quotient of 3.02 is comparable with that in shoots of rice (3.56 mg Mg g^{-1} DM; Zeng *et al.*, 2010). These values suggest that at least 18% of leaf Ca and 3% of leaf Mg by mass will be present in the cell walls of monocots. Given the lack of precision in the estimates of the volumes of cellular compartments within leaves, total Ca and Mg concentrations in cellular compartments, leaf DM/FW quotient and the contribution of the cell wall to leaf biomass, the prediction is remarkably concordant with measured values. From these observations, the relationship between $[Mg]_{shoot}$ and $[Ca]_{shoot}$ among commelinid monocots (**Figure 1a**) can then be predicted assuming a cell wall Ca/Mg mass quotient of 4.00 using the equation $[\mathrm{Mg}]_{\mathrm{shoot}} = 1.75 + (0.25 \times [\mathrm{Ca}]_{\mathrm{shoot}})$. Assuming similar intracellular Ca and Mg concentrations in all angiosperms, the relationship between $[\mathrm{Mg}]_{\mathrm{shoot}}$ and $[\mathrm{Ca}]_{\mathrm{shoot}}$ among eudicots excluding Caryophyllales species (**Figure 1b**) can be predicted assuming a cell wall Ca/Mg mass quotient of 10.0 using the equation $[\mathrm{Mg}]_{\mathrm{shoot}} = 1.90 + (0.10 \times [\mathrm{Ca}]_{\mathrm{shoot}})$. These predictions fit the data reasonably well, although the predictions of $[\mathrm{Mg}]_{\mathrm{shoot}}$ for monocots are generally greater than the observed $[\mathrm{Mg}]_{\mathrm{shoot}}$ at a given $[\mathrm{Ca}]_{\mathrm{shoot}}$, suggesting that intracellular Mg might be overestimated in monocots, and that intracellular Mg might be less in commelinid monocots than in eudicots (**Figure 1a,b**).

White *et al.* (2015) suggested that Caryophyllales species have larger $[\mathrm{Mg}]_{\mathrm{shoot}}$ and smaller shoot Ca/Mg quotients than other angiosperms because of greater accumulation of Mg in vacuoles of shoot cells. The accumulation of Mg, but the same amount of Ca, in a vacuole can give rise to both phenomena if all other factors remain equal and produce the relationship between $[\mathrm{Ca}]_{\mathrm{shoot}}$ and $[\mathrm{Mg}]_{\mathrm{shoot}}$ among Caryophyllales species observed in the dataset analysed here (**Figure 1b**) as well as in previous studies (Thompson *et al.*, 1997; Broadley *et al.*, 2004; White *et al.*, 2012, 2015). Assuming that the Mg concentration in other cellular compartments remains equal, and that the Mg concentration in the cell wall of a plant with a shoot Ca concentration of 1 mg kg^{-1} is 0.12 mg Mg g^{-1} cell wall DM, then the vacuolar Mg concentration in this plant can be calculated to be 14.19 mM (**Table 1**). The relationship between $[\mathrm{Mg}]_{\mathrm{shoot}}$ and $[\mathrm{Ca}]_{\mathrm{shoot}}$ in Caryophyllales can be predicted assuming a cell wall Ca/Mg mass quotient of 5.88, using the equation $[\mathrm{Mg}]_{\mathrm{shoot}} = 4.68 + (0.17 \times [\mathrm{Ca}]_{\mathrm{shoot}})$.

CONCLUSIONS AND PERSPECTIVE

This article presents a novel, quantitative and universal explanation of the differences in shoot Ca/Mg quotients and absolute Ca and Mg concentrations in the shoots of angiosperm species. The arguments and analysis presented lead to several hypotheses, namely, that in plants that do not exhibit "luxury" accumulation of Ca or Mg, (1) distinct linear relationships between $[Mg]_{shoot}$ and $[Ca]_{shoot}$ are exhibited by at least three groups of angiosperm species, namely commelinid monocots, eudicots excluding Caryophyllales, and Caryophylalles species, (2) that these relationships are determined by cell wall chemistry and the Mg/Ca mass quotients in their cell walls, (3) that differences between species in $[Ca]_{shoot}$ and $[Mg]_{shoot}$ within groups are associated with their cell wall CEC, and (4) that Caryophyllales constitutively accumulate more Mg in their vacuoles than other angiosperm species.

These hypotheses might be tested through further experimentation. The hypothesis that different groups of angiosperm species exhibit distinct linear relationships between $[Mg]_{shoot}$ and $[Ca]_{shoot}$ might be tested by surveying the shoot ionomes of more species within each group. Similarly, the hypothesis that the relative concentrations of Ca and Mg in shoot cell walls differ between groups of angiosperm species and correlate with the gradient of their $[Mg]_{shoot}$ and $[Ca]_{shoot}$ relationships might be tested by determining the cationic composition of shoot cell walls of more plant species from each group. The hypothesis that the absolute Ca and Mg concentrations in shoot cell walls of species within each group are determined by their CEC might be tested by assaying the cell wall Ca and Mg concentrations and CEC of shoots of more plant species from each group. The role of particular cell wall compounds in determining CEC and the absolute and relative concentrations of Ca and Mg in the cell wall might be tested using mutants with less or more of these compounds. The greater accumulation of Mg in the vacuole of Caryophyllales might be tested by comparing Mg localisation at sub-cellular resolution in shoots of species from different angiosperm orders grown under identical conditions.

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Table 1. Data used to predict shoot calcium concentrations ($[\mathrm{Ca}]_{\mathrm{shoot}}$) and shoot magnesium concentrations ($[\mathrm{Mg}]_{\mathrm{shoot}}$) of angiosperm species.

	Cell Compartment						
	Cell Wall	Cytosol	ER	Nucleus	Mitochondria	Chloroplasts	Vacuoles
[1] Volume (% Leaf)[a]							
Angiosperms	6.0	5.0	0.5	0.5	1.0	5.0	82
[2] Calcium Concentration (mM)[b]							
Angiosperms		0.1	2	2	2	7	2
[3] Magnesium Concentration (mM)[c]							
Angiosperms except Caryophyllales		2	10	10	7	105	3
Caryophyllales		2	10	10	7	105	14
[4] Ca/Mg quotient in material above minimal cell wall (g/g)[d]							
Commelinids	4.00						
Eudicots except Caryophyllales	10.0						
Caryophyllales	5.88						

[a] Heldt and Piechulla (2010), Hawkesford *et al.* (2012)

[b] Carr *et al.* (2003), White and Broadley (2003), Stael *et al.* (2012)

[c] Shaul (2002), Carr *et al.* (2003), Hawkesford *et al.* (2012), Gout *et al.* (2014)

[d] Gradients of the relationships between $[\mathrm{Mg}]_{\mathrm{shoot}}$ and $[\mathrm{Ca}]_{\mathrm{shoot}}$ presented in **Figure 1a,b**

Figures

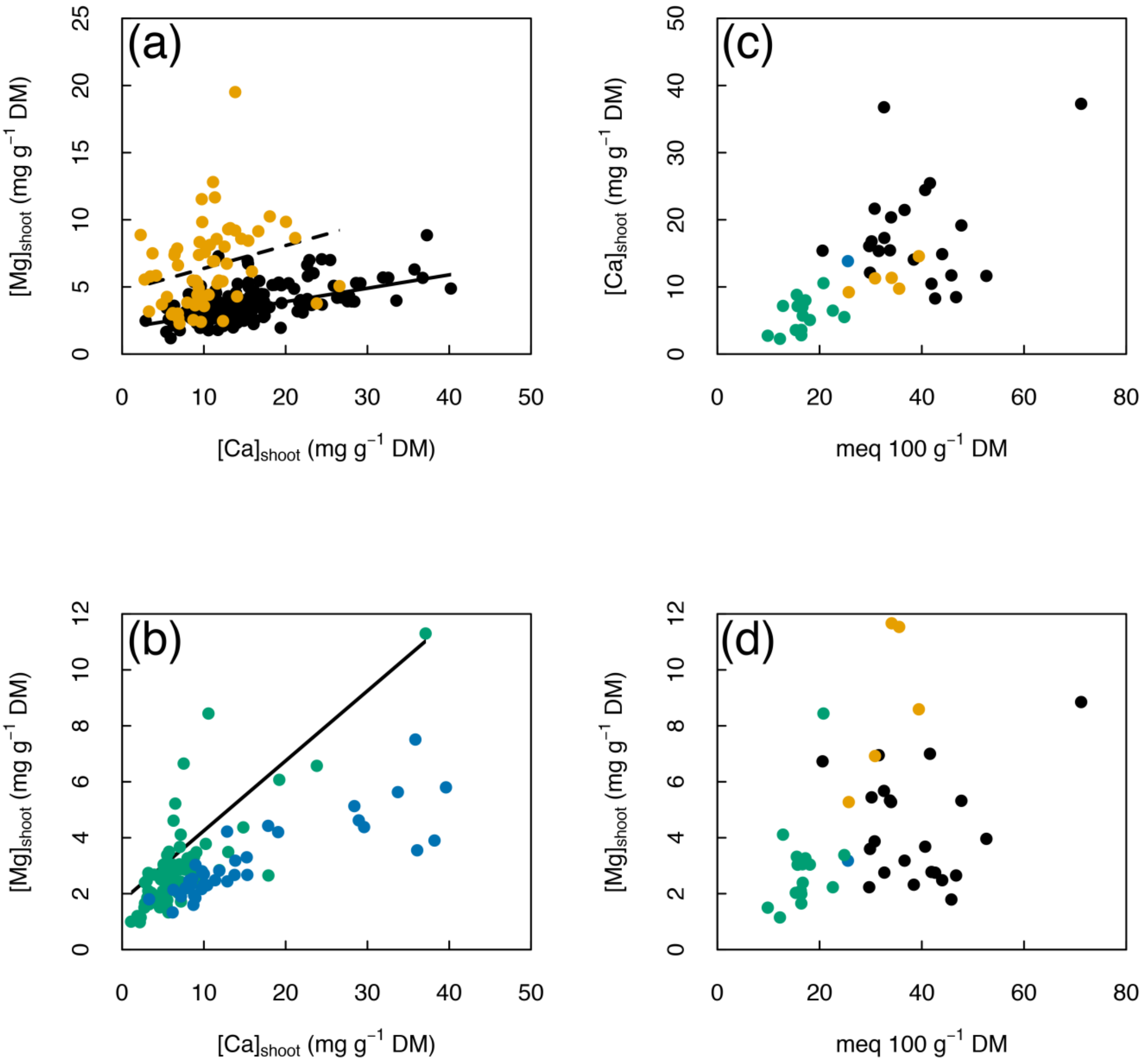
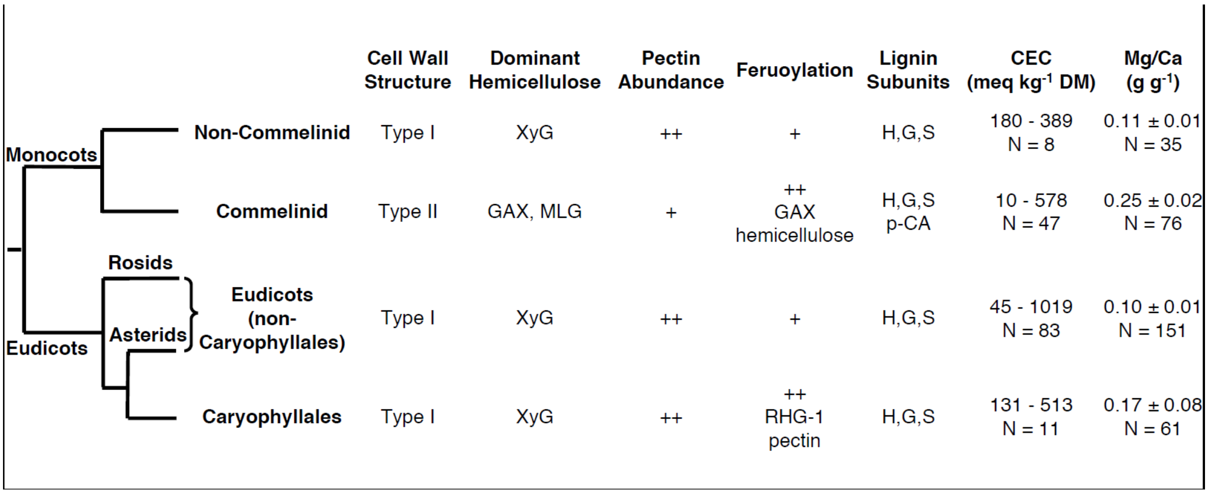


Figure 1. (a) The relationship between shoot Mg concentration ($[Mg]_{shoot}$) and shoot concentration ($[Ca]_{shoot}$) among 212 eudicot species (black and orange circles), of which 61 were members of the Caryophyllales order (orange circles). The lines are predictions for the relationships between $[Mg]_{shoot}$ and $[Ca]_{shoot}$ for non-Caryophyllales eudicots (solid line) and Caryophyllales species (broken line) based on the model based on leaf anatomy described in the text and the data presented in Table 1. **(b)** The relationship between $[Mg]_{shoot}$ and $[Ca]_{shoot}$

among 76 commelinid (green circles) and 35 non-commelinid monocot species (blue circles). The original dataset contained 3 non-commelinid species with $[Ca]_{shoot} > 50$ mg g^{-1} DM that are not plotted. The line is a prediction for the relationship between $[Mg]_{shoot}$ and $[Ca]_{shoot}$ for commelinid monocots based on the model based on leaf anatomy described in the text and the data presented in Table 1. **(c)** The relationship between $[Ca]_{shoot}$ and the cation exchange capacity (CEC) of root cell walls of 44 angiosperm species, comprising 16 commelinid monocots (green circles), 1 non-commelinid monocot (blue circle), 5 Caryophyllales (orange circles) and 22 other eudicots (black circles). **(d)** The relationship between $[Mg]_{shoot}$ and the CEC of root cell walls of the same 44 angiosperm species. The $[Ca]_{shoot}$ and $[Mg]_{shoot}$ for angiosperm species are mean values obtained in the six hydroponic experiments described by White *et al.* (2017) and collated by Neugebauer *et al.* (2018). Values for the CEC of root cell walls were obtained from the literature survey of White and Broadley (2003) and are means from a REML statistical analysis of the data.



	Cell Wall Structure	Dominant Hemicellulose	Pectin Abundance	Feruoylation	Lignin Subunits	CEC (meq kg^{-1} DM)	Mg/Ca (g g^{-1})
Non-Commelinid	Type I	XyG	++	+	H,G,S	180 - 389 N = 8	0.11 ± 0.01 N = 35
Commelinid	Type II	GAX, MLG	+	++ GAX hemicellulose	H,G,S p-CA	10 - 578 N = 47	0.25 ± 0.02 N = 76
Eudicots (non-Caryophyllales)	Type I	XyG	++	+	H,G,S	45 - 1019 N = 83	0.10 ± 0.01 N = 151
Caryophyllales	Type I	XyG	++	++ RHG-1 pectin	H,G,S	131 - 513 N = 11	0.17 ± 0.08 N = 61

Figure 2. Phylogenetic relationships between non-commelinid monocots, commelinid monocots, eudicots excluding Caryophyllales (rosids and asterids) and Caryophyllales according to the Angiosperm Phylogeny Group (APGIV, 2016). The presence of Type I (cellulose microfibrils surrounded by xyloglucan [XyG] with large amounts of pectin; lignin containing H [p-hydroxyphenyl], G [guaiacyl] and S [syringyl] subunits) or Type II (cellulose microfibrils surrounded by glucuronoarabinoxylan [GAX] and some mixed linkage glucans [MLG], with little pectin; lignins containing H, G, S and ester-related p-coumaric acid [p-HCA] subunits) cell walls, dominant hemicelluloses, pectin abundance (++ = large amounts, + = small amounts), feruoylation (++ = large amounts, + = small amounts), and lignin subunits are indicated. Data for cell wall composition is summarised from Harris and Trethewey (2010), Popper et al., (2011), Banasiak (2015) and Hatfield et al., (2017). Root cell wall cation exchange capacities (CEC, expressed as the range for N species) are taken from White and Broadley (2003) and gradients of the relationships between $[Mg]_{shoot}$ and $[Ca]_{shoot}$ are derived from the data shown in **Figure 1a,b**.