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Seagrass leaf element content: A global overview

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ABSTRACT

Knowledge on the role of seagrass leaf elements and in particular micronutrients and their ranges is limited. We present a global database, consisting of 1126 unique leaf values for ten elements, obtained from literature and unpublished data, spanning 25 different seagrass species from 28 countries. The overall order of average element values in seagrass leaves was Na > K > Ca > Mg > S > Fe > Al > Si > Mn > Zn. Although we observed differences in leaf element content between seagrass families, high intraspecific variation indicated that leaf element content was more strongly determined by environmental factors than by evolutionary history. Early successional species had high leaf Al and Fe content. In addition, seagrass leaf element content also showed correlations with macronutrients (N and P), indicating that productivity also depends on other elements. Expected genomes of additional seagrass species in combination with experiments manipulating (micro)nutrients and environmental drivers might enable us to unravel the importance of various elements to sustain productive and flourishing meadows.

1. Introduction

The functions of elements in seagrasses have been examined in various contexts across the world. Seagrass elemental composition has been intensively studied for the macronutrients nitrogen (N) and phosphorus (P) as drivers for productivity and in relation to nutrient limitation and disturbances such as eutrophication (e.g. Duarte, 1990; Fourqurean and Zieman, 2002). Metal content such as copper, nickel and lead have been studied in relation to metal accumulation and the role of seagrass in predicting water quality as bioindicator (Prange and Dennison, 2000; Govers et al., 2014b; Bonanno and Di Martino, 2016). The potential importance of some other nutrients for the productivity and nutrient dynamics of seagrasses has been acknowledged (e.g. for Fe; Romero et al., 2006; Van Tussenbroek et al., 2006), but has only been sparsely investigated (e.g. Duarte et al., 1995; Van Katwijk et al., 2011).

Elements, and micronutrients in particular, have various known functions in plant physiology and metabolism (see Marschner, 2011 for an extensive overview). For seagrasses, knowledge on the functions of various elements besides N and P that may act as nutrients is more limited, yet, several studies have reported on some of these functions (Table 1). Like in all submerged macrophytes, potassium (K), sodium

(Na) and calcium (Ca) are especially important for osmosis regulation (e.g., Touchette, 2007; Olsen et al., 2016). Magnesium (Mg) and iron (Fe) are essential elements involved in photosynthesis, and while Mg is generally abundantly available in seawater (Pilson, 2013), Fe availability can be limited especially in carbonate sediments (Duarte et al., 1995). Zinc (Zn) is involved in protein synthesis (Malea et al., 1995) and manganese (Mn) is important in various redox processes (Jagtap, 1983). Sulphur (S) is used in cell walls as part of sulphated polysaccharides (Olsen et al., 2016) and can accumulate in seagrass tissue (Holmer et al., 2005). Finally, leaf aluminium (Al) and silicon (Si) content have been reported (Malea, 1993; Herman et al., 1996), although no specific functions for these elements are known in seagrasses.

In general, plant stoichiometry is highly variable, plastic, and dependent on the environment (Sterner and Elser, 2002; Marschner, 2011). Plant element ratios closely reflect dominant environmental conditions and potential mechanistic adaptations of how plants deal with adverse environmental conditions (Ågren and Weih, 2012). For example, like all vascular plants growing in seawater, seagrasses have to deal with a medium that contains high ion concentrations. Salt tolerant plants have active uptake mechanisms for certain elements to withstand osmotic pressure and/or to excrete elements that negatively influence cell functioning (Flowers et al., 1977; Subbarao et al., 2003;

Table 1
Overview of known functions of the studied elements in seagrasses.

Element	Functions in seagrasses	References
K	Elevated levels of K in seagrass tissue balance the osmotic potential in order to prevent dehydration in a saline environment.	Touchette, 2007
Na	Na is elevated in seagrasses, primarily in the vacuoles, to maintain the osmotic potential. At high environmental concentrations Na can replace K for binding sites, so antiporters in the membrane minimize Na accumulation.	Carpaneto et al., 1997; Touchette, 2007; Olsen et al., 2016
Ca	Calcium carbonate is produced by calcifying epiphytes on the seagrass leaves, and by the seagrass itself both internally in the cell wall and on the leaf surface. Calcium is also involved in osmoregulation by reducing Na uptake and increasing K and Ca uptake.	Marbà et al., 2006; Hadi and Karimi, 2012; Enríquez and Schubert, 2014
Mg	Mg is the central atom of the chlorophyll molecule, and therefore essential in photosynthesis. Mg is generally not limiting in seagrass ecosystems. High environmental concentrations can become toxic.	Marschner, 2011; Thangaradjou et al., 2013
S	Toxic sulphide in marine sediments is re-oxidized by seagrass and can be accumulated as elemental sulphur in their tissue. Sulphated polysaccharides in the cell wall facilitate ion and water retention.	Holmer et al., 2005, 2009; Olsen et al., 2016
Fe	Important in electronic transport chain for photosynthesis, about 80% of the total iron is localized in chloroplasts, in case of deficiency PS I drops first. Fe can become limiting especially in tropical carbonate sediments.	Duarte et al., 1995; Marschner, 2011;
Mn	Important in redox processes because of the high number of oxidation states, toxic effects to seagrasses are unknown.	Jagtap, 1983; Thangaradjou et al., 2013
Zn	Important in protein synthesis, high concentrations of Zn can induce seagrass leaf mortality.	Malea et al., 1995; Marschner, 2011;
Al	Seagrasses are commonly unaffected by toxic Al^{3+} concentrations because they have a cytoplasmatic pH above 6.0 at which Al is harmless.	Thake et al., 2003
Si	Unknown, but reported limiting in environments where terrestrial runoff is lacking. Si improves the rigidity of macrophytes in dynamic environment and could be involved in salt tolerance.	Herman et al., 1996; Liang et al., 2007; Schoelynck and Struyf, 2016

Hadi and Karimi, 2012). Adaptations of seagrasses to marine conditions might be correlated to evolutionary lineages. Seagrasses made the transition from freshwater to saltwater environments at least three times during evolutionary history (78–40 million years ago) and the four seagrass families together thus form an ecological group rather than a monophyletic clade (Les et al., 1997; Den Hartog and Kuo, 2006). Recent research to the complete genome of *Zostera marina* (Zosteraceae) revealed some of the genes responsible for the physiological and structural adaptations for its return to sea (Olsen et al., 2016). Members from the other seagrass lineages can have evolved similar physiological adaptations, however, differences in leaf element content can be indicative that other lineages have developed alternative mechanisms to flourish under these challenging conditions.

To investigate the role and importance of (micro)nutrients for seagrass physiology and functioning we aimed to provide a global overview of the leaf elemental content (Al, Ca, Fe, K, Mg, Mn, Na, S, Si, Zn) of seagrasses. We therefore collected data for species that belong to the four seagrass families (Zosteraceae, Cymodoceaceae, Posidoniaceae and Hydrocharitaceae) and discuss the potential role of these (micro)nutrients in seagrass physiology and their role in adaptations of seagrass species through evolutionary history. Data were collected from both literature and unpublished field datasets. Since seagrass species distribution often covers a substantial amount of the globe and plants are exposed to a high variety of environmental conditions (e.g. Green and Short, 2003), we hypothesized that environmental conditions drive seagrass element content and expect large variation within and between species. Evolutionary adaptations were expected to be reflected by clear differences in leaf element content between seagrass families (e.g. Broadley et al., 2004). Additionally, differences in leaf element content in species of different successional stage (i.e. climax and colonizer species) could indicate the importance of elements for seagrass productivity. Finally, relations between leaf elements were derived from correlations between the ten studied elements and between these elements and the macronutrients N and P.

2. Materials and methods

2.1. Literature search

Seagrass leaf element data were collected from Web of Science (ISI; search: "seagrass" AND (Sodium OR Potassium OR Calcium OR Magnesium OR Sulphur OR Silicon OR Iron OR Zinc OR Aluminium OR Manganese)) and Google Scholar©, accessed Dec. 2016. We collected element contents derived from graphs, tables and text of 84 studies (see Supplement 1). Measurements from the same study were averaged if sampled < 1 km apart (measured in Google maps©), if located in the same bay, or if sampled at different seasons. Seagrasses growing in known polluted or impacted sites have been omitted from our database and only natural or reference location have been included. Only leaf element content was used and all values were converted to % dry weight of leaf, yielding in total 605 unique data points. Data included in our database spanned a 40-year period, from 1975 up to 2016. The constructed literature database consisted of seagrass habitats on all continents where seagrasses occur (all but Antarctica); ranging from 59° northern to 36° southern latitudes (Fig. 1). Literature data was obtained for 25 different seagrass species from 28 countries.

2.2. Leaf element analysis

In addition to obtaining data on leaf elemental content from literature, we analysed leaf elemental content of field samples, including nitrogen, phosphorus and carbon (C). In total, 318 leaf samples were collected from twenty locations spanning nine different countries and twelve species belonging to three seagrass families (Table S4). Leaves were carefully cleaned from epiphytes, rinsed and dried (60 °C, 48 h). Dried material was transported back to the Netherlands and ground. Prior to elemental analyses, samples were digested in pressure tubes using H_2O_2 and HNO_3 in an autoclave for 30 min at 121 °C as described by Smolders et al. (2006). Total concentrations of Al, Ca, Fe, K, Mg, Mn, Na, S, Si, Zn and P in seagrass leaf tissue were measured by inductively coupled plasma emission spectrometry (ICP-MS, IRIS Intrepid II, Thermo Electron Corporation, Franklin, USA). Leaf C and N content in this ground leaf material was analysed using an elemental analyser (Type NA 1500 Carlo Erba Thermo Fisher Science, USA), coupled online via an interface (Finnigan Conflo III) to a mass spectrometer (Thermo Finnigan Delta Plus, USA).

2.3. Constructed databases

We constructed a *global seagrass leaf element database* (Supplement 1) based on obtained literature values and from analysed leaf elements. We merged analysed elements of seagrass leaf samples from nearby locations (< 1 km) before adding these average values to the global database (reference "authors' data" in Supplement 1). The data points
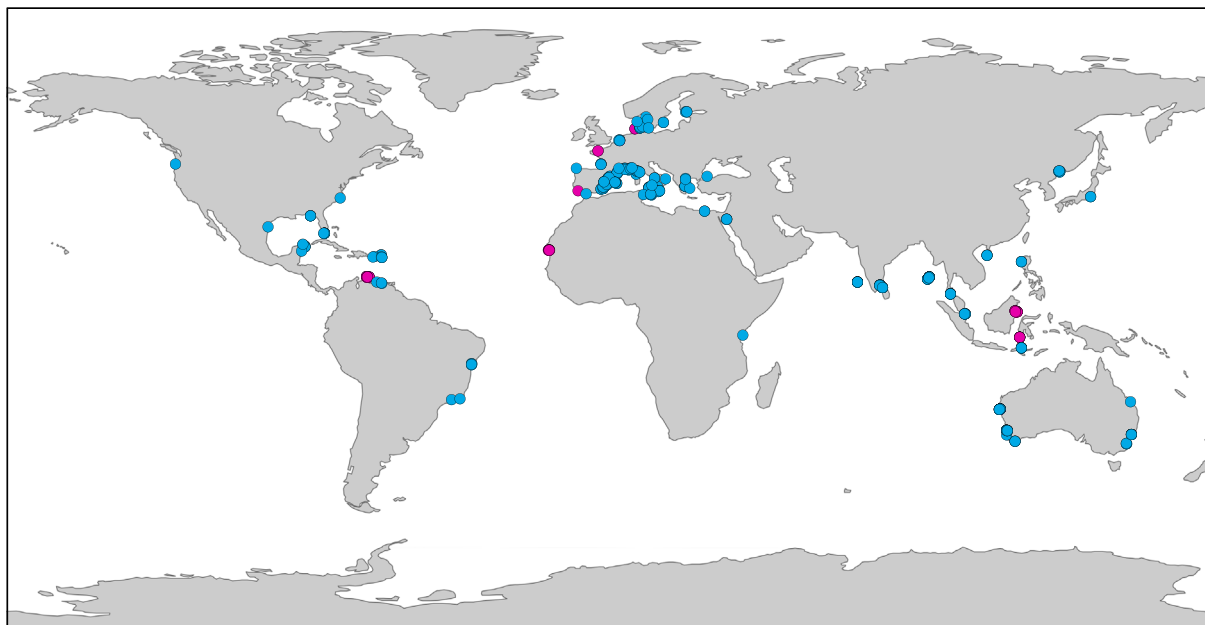
Fig. 1. Global overview of seagrass leaf element sampling locations. Sampling locations by the authors (pink, n = 9) were supplemented with literature data (blue, n = 84) to construct our global database. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in our database are well spread throughout the global seagrass home ranges and represent a wide range in species (overview in Table S2).

Secondly, we constructed a seagrass *leaf element correlation matrix* (Supplement 2) comprising of all 318 individually measured leaf element samples (accounting in total for 3073 separate values of leaf Al, Ca, Fe, K, Mg, Mn, Na, S, Si, or Zn content; Table S5), and included measured C (287), N (290) and P (292) values. Furthermore, we added information, if available, on local conditions where the leaf samples were collected.

2.4. Statistics

Using the *global seagrass leaf element database*, differences in leaf element content between families, species and successional stages were tested with Linear Mixed Models (LMM) with either "family", "species", or "successional stage" as fixed factors and "location" as a random factor, to be able to nest multiple samples per location in the model.

From the *leaf element correlation matrix* (Supplement 2), we assessed leaf element content in a mixed-species seagrass meadow on Derawan, Indonesia. All three species, *Halodule uninervis*, *Syringodium isoetifolium* and *Thalassia hemprichii*, were exposed to comparable environmental conditions, we used this community to determine interspecific effects on leaf element content using Linear Models (LM) with "species" as

fixed effect. Since *Zostera noltii* samples were collected on four locations almost across its entire east-Atlantic range, from West Africa (Mauritania), Brittany (France), Oosterschelde (the Netherlands), to Sylt (Germany), we used this species to determine intraspecific effects on leaf element content using LMs with "country" as fixed effect. Correlations between seagrass leaf elements and also between the seagrass leaf elements and the macronutrients N and P were analysed using Spearman's rank correlations tests.

Model assumptions were tested on model residuals using a Shapiro test and judgement of histograms of model residuals. Data were log-transformed if necessary to meet model assumptions. All statistical analyses were performed in R version 3.3.2 "Sincere Pumpkin Patch" © 2016, The R foundation for Statistical Computing.

3. Results

3.1. Overview global database

For all ten elements, representing a wide range of potential functions in seagrass physiology (Table 1), we obtained seagrass leaf element content, expressed as percentage dry weight (Table 2). Based on our global database, seagrass leaves contained nutrients in the overall following order: Na > K > Ca > Mg > S > Fe > Al > Si >

Table 2
Overview of the global seagrass leaf element database as characteristics per element, includes all literature values and author's values (mean, SE, min and max are in % DW). Estimates are unique combinations of species and location.

| Element	Estimates	Species	Countries	Locations	Leaf element content			
	#	#	#	#	Mean	SE	Min	Max
Al	96	16	10	44	0.084	0.016	$4.0E - 04$	1.062
Ca	74	17	16	30	1.921	0.176	$2.0E - 03$	8.086
Fe	181	22	24	97	0.115	0.020	$2.2E - 05$	2.913
K	81	18	16	32	2.008	0.152	$1.0E - 03$	7.194
Mg	84	19	15	33	0.949	0.075	$9.0E - 03$	4.632
Mn	145	20	19	78	0.029	0.004	$2.4E - 05$	0.233
Na	69	17	15	30	5.078	0.491	$3.0E - 03$	22.345
S	103	19	13	44	0.771	0.049	$3.0E - 03$	3.646
Si	57	13	9	23	0.082	0.012	$2.0E - 02$	0.460
Zn	236	22	26	135	0.009	0.001	$5.0E - 06$	0.159
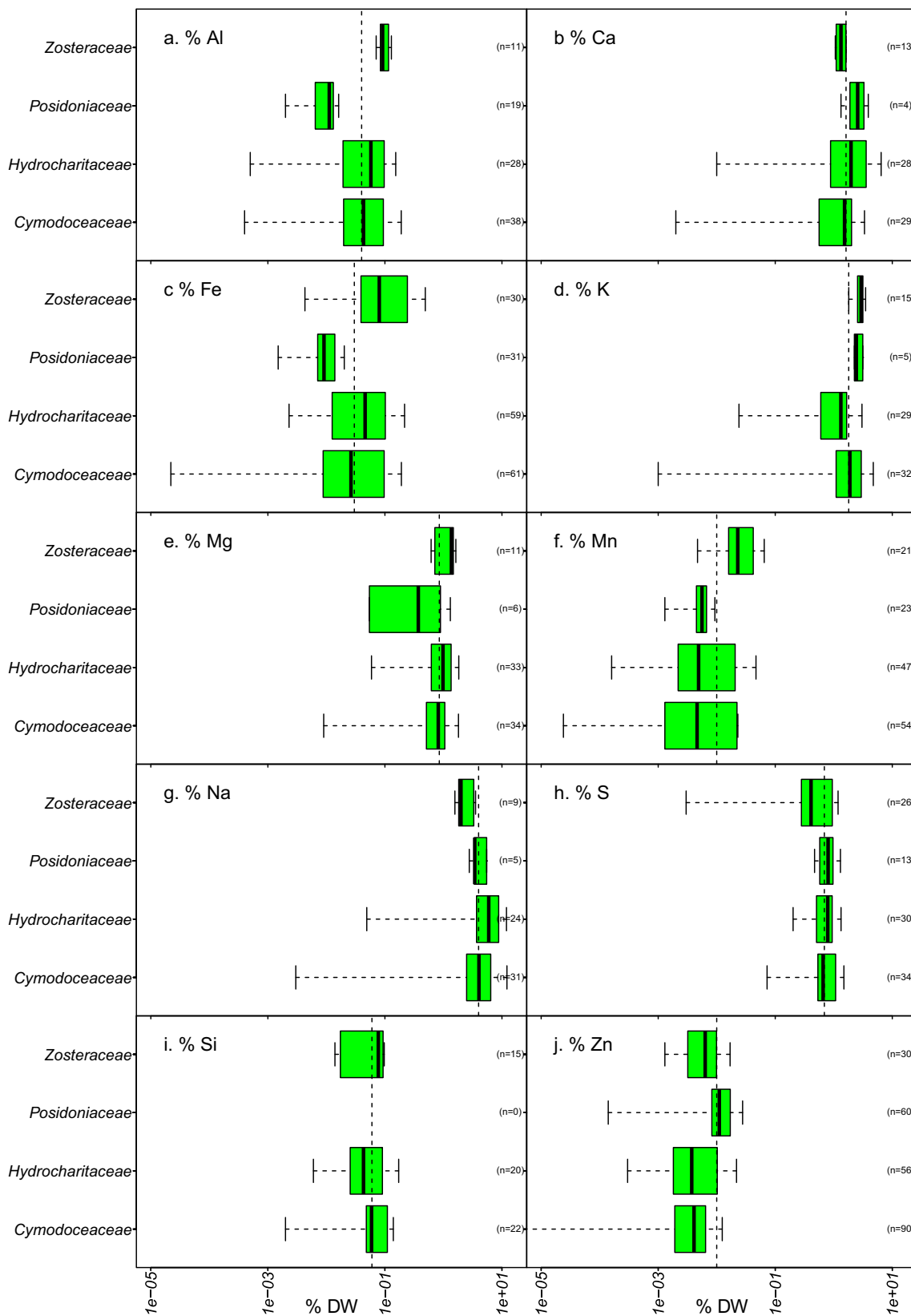
Fig. 2. Seagrass leaf (micro)nutrient content of the four seagrass families: Zosteraceae, Posidoniaceae, Hydrocharitaceae and Cymodoceaceae. Elemental content of a) aluminium (Al), b) calcium (Ca), c) iron (Fe), d) potassium (K), e) magnesium (Mg), f) manganese (Mn), g) sodium (Na), h) sulphur (S), i) silicate (Si) and j) zinc (Zn) was determined as % of dry weight (DW) in samples from seagrass leaves. Straight dotted lines indicate the median element values of all samples and bold lines within boxplots indicate median element content per seagrass family. Unique sample numbers (counts) are indicated behind the boxes.

Mn > Zn, with individual values ranging from 0.000005% DW Zn to 22.34% DW Na. The elements Na, K, Ca, S and Mg often occurred in percentages two orders of magnitude greater than Fe, Al, Si, Mn and Zn in seagrass leaves. Lowest variation among seagrasses was observed for leaf S and Si content, while leaf Mg content had the lowest variation of all metals. Overall, leaf Fe content had the highest reported variation, ranging from 0.000022% DW to 2.91% DW. In total, we had the lowest amount of observations on Si (57) whereas Zn was most studied (236 observations). The distribution of the leaf element contents was often left-skewed (Fig. S1).

3.2. Comparison of leaf elements between families

Leaf element content varied substantially among the four seagrass families for seven out of ten studied elements (Fig. 2). Only for K (LMM, $F_{3,46} = 1.051$, $p = 0.379$), Na (LMM, $F_{3,36} = 2.322$, $p = 0.0915$) and Si (LMM, $F_{2,32} = 0.601$, $p = 0.555$) no significant differences in leaf content were observed between families. Posidoniaceae leaf content for both Al (Fig. 2a, LMM, $F_{3,49} = 9.804$, $p < 0.001$) and Zn (Fig. 2j, LMM, $F_{3,98} = 13.090$, $p < 0.001$) significantly differed from all other families (on average $10 \times$ lower leaf Al content and $2 \times$ higher leaf Zn content). For the other elements, patterns were less distinct. For instance, leaf Fe content only differed significantly between Zosteraceae and Posidoniaceae, with on average almost $9 \times$ lower Fe levels in Posidoniaceae than in Zosteraceae (Fig. 2c, LMM, $F_{3,80} = 3.282$, $p = 0.025$). We observed a similar trend for Mg (Fig. 2e, LMM, $F_{3,48} = 3.332$, $p = 0.027$), with $2.2 \times$ lower Mg content in Posidoniaceae than in Zosteraceae. Zosteraceae had up to $6.0 \times$ higher leaf Mn content (LMM, $F_{3,64} = 3.705$, $p = 0.016$). up to $1.5 \times$ lower leaf S content (Fig. 2h, LMM, $F_{3,55} = 6.283$, $p < 0.001$) compared to all other families. Finally, leaf Ca content differed only significantly between Hydrocharitaceae and Cymodoceaceae, with on average $1.7 \times$ lower Ca content in Cymodoceaceae than in Hydrocharitaceae (Fig. 2b, LMM, $F_{3,41} = 3.733$, $p = 0.018$).

3.3. Interspecific variation

Similar to the above analyses at family level, leaf content was comparable for Ca (LMM, $F_{16,28} = 1.733$, $p = 0.098$), K (LMM, $F_{17,32} = 1.6879$, $p = 0.099$), and Na (LMM, $F_{16,23} = 1.373$, $p = 0.238$) while we found significant differences between species for all seven other elements (Fig. 3). Leaf Al content (Fig. 3a, LMM, $F_{15,37} = 5.361$, $p < 0.001$) in *Posidonia oceanica* was on average $> 8 \times$ lower than the overall mean value and even up to $100 \times$ lower compared to *Halophila ovalis*, *Zostera noltii*, and *Thalassia testudinum*. Leaf Fe content (Fig. 3c, LMM, $F_{21,61} = 4.3295$, $p < 0.001$) was especially high in *Zostera* and *Halophila* species, with average values $1.5 \times$ and $5.2 \times$ higher than the average leaf Fe content, respectively. Leaf Mg content (Fig. 3f, LMM, $F_{18,33} = 2.821$, $p = 0.005$) was $16 \times$ lower in *Posidonia australis* compared to the average leaf Mg content. Leaf Mn content (Fig. 3g, LMM, $F_{20,48} = 4.8723$, $p < 0.001$) was high in *Halophila decipiens* (4.4 x higher than average) and very low in *Cymodocea serrulata* ($53 \times$ lower than average). Leaf S content (Fig. 3h, LMM, $F_{18,40} = 2.53$, $p = 0.007$) was $2.7 \times$ lower in *Zostera marina*, than the average S content. Leaf Si (Fig. 3i, LMM, $F_{12,22} = 14.369$, $p < 0.001$) was surprisingly low in *Zostera noltii* ($8.6 \times$ lower than average), a species that is generally growing in high Si environments (terrigenous sediments). Finally, leaf Zn (Fig. 3j, LMM, $F_{21,80} = 6.562$, $p < 0.001$) was on average $1.6 \times$ higher in *Posidonia oceanica* compared to all other species.

3.4. Differences between successional stages

Patterns in leaf element content between species were also reflected in differences between seagrass successional stages (Fig. 4). Colonizing species had significant higher leaf content than climax species for Al (LMM, $F_{1,51} = 24.31$, $p < 0.001$; $0.121 \pm 0.026\%$ DW Al and

$0.034 \pm 0.009\%$ DW Al, respectively), for Fe (LMM, $F_{1,82} = 11.87$, $p < 0.002$; $0.175 \pm 0.033\%$ DW Fe and $0.033 \pm 0.010\%$ DW Fe, respectively), for Mn (LMM, $F_{1,66} = 4.41$, $p = 0.039$) and for Si (LMM, $F_{1,33} = 5.28$, $p = 0.028$). Leaf Zn content of colonizing species was significantly lower compared to climax species (LMM, $F_{1,101} = 4.33$, $p = 0.040$; $0.0083 \pm 0.0014\%$ DW Zn and $0.0109 \pm 0.0015\%$ DW Zn, respectively), while leaf Ca, K, Mg, Na, and S content were comparable between seagrass successional stages (all $p > 0.05$).

3.5. Within community variation

Analysing within community variation in leaf element content from a mixed-species seagrass meadow on Derawan (Indonesia) showed that, besides for Ca (LM, $F_{3,69} = 7.637$, $p < 0.001$) and Na (LM, $F_{3,69} = 4.174$, $p = 0.009$), leaf content was comparable for the other studied elements (LM, $p > 0.05$; Fig. S2). *Thalassia hemprichii* ($4.19 \pm 0.75\%$ DW) had higher leaf Ca content compared to *Halodule uninervis* ($1.52 \pm 0.18\%$ DW) and *Syringodium isoetifolium* ($1.80 \pm 0.62\%$ DW). Leaf Na content was significantly higher in *T. hemprichii* ($16.3 \pm 2.8\%$ DW) compared to *H. uninervis* ($6.9 \pm 1.2\%$ DW), with intermediate values for *S. isoetifolium* ($12.1 \pm 3.4\%$ DW).

3.6. Intraspecific variation

Analysing intraspecific leaf element content of the intertidal species *Zostera noltii* showed that besides for Zn (LM, $F_2 = 1.43$, $p = 0.249$) all other elements showed intraspecific variation between countries (Fig. S3). Leaf Fe content (LM, $F_2 = 62.0$, $p < 0.001$) was $> 4 \times$ lower in Mauritania than in France and the Netherlands. In contrast, leaf K content (LM, $F_3 = 4.60$, $p = 0.006$) was $1.3 \times$ higher in Mauritania than in France. For leaf Mg content (LM, $F_2 = 364$, $p < 0.001$) and leaf Mn content (LM, $F_2 = 106$, $p < 0.001$), we found very high intraspecific variation as all countries differed significantly. *Z. noltii* leaf Mg content was lowest in the Netherlands (0.6%) and highest in Mauritania (1.5%), while leaf Mn content was highest in the Netherlands (0.17%) and lowest in Mauritania (0.02%). Leaf S content (LM, $F_2 = 18.6$, $p < 0.001$) was significantly higher (up to $1.2 \times$) in Mauritanian *Z. noltii* than in all other countries. In contrast, Si content (LM, $F_2 = 178$, $p < 0.001$) in Mauritania was significantly lower than in all other countries ($< 5 \times$). Variations in leaf Al, Ca, and Na content were more subtle (Fig. S3).

3.7. Correlations between leaf elements

Besides leaf Mn content, all other studied seagrass leaf elements showed correlations, with in total 19 significant correlations out of possibly 45 for all families together (Fig. S4). Most correlations were observed for leaf Ca content (five other elements: Al, K, Mg, Na, and S) and leaf Al content (four other elements: Ca, Fe, K, and Na). Especially the elements K, Na, Mg and S showed strong positive correlations (all $r > 0.5$; Fig. 5), however, there were large differences between different families. These correlations were observed most strongly for the Hydrocharitaceae (all $r > 0.82$). Correlations for K with the other three elements were less strong for the Cymodoceaceae, while for the Zosteraceae only two correlations (Mg to Na and Mg to S) were detected.

3.8. Correlations between leaf elements and macronutrients

Correlations between leaf nitrogen content and the studied leaf element content for all seagrasses together showed only very weak significant relationships (all $r < |0.4|$; Fig. S5), with positive correlations between leaf N and leaf Ca, Fe, Mg, Mn, S and Si content, and negative correlations between leaf N and leaf K content. However, when we distinguished between families, strong correlations ($r > |0.7|$) between seagrass leaf N and element contents were
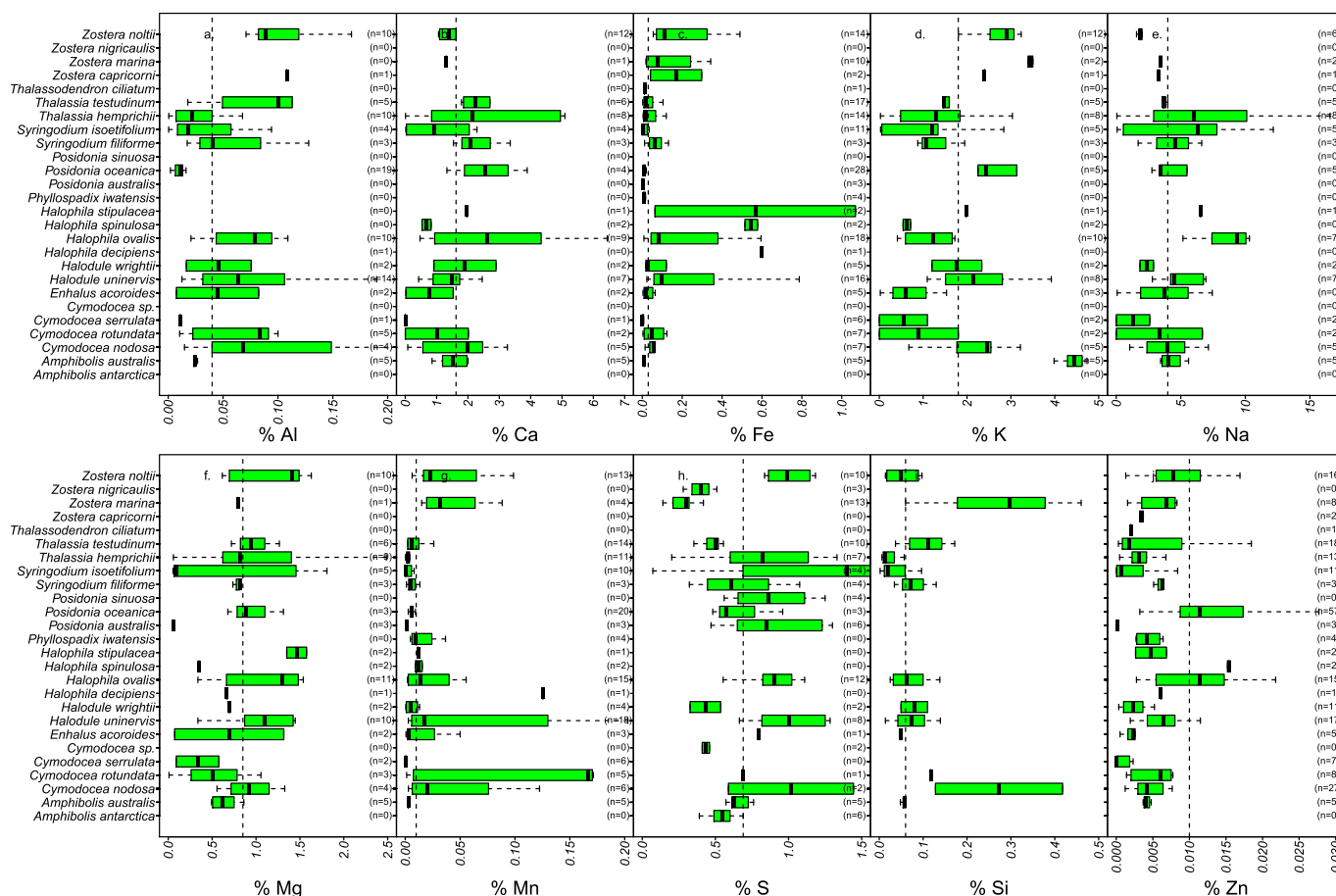
Fig. 3. Overview of leaf (micro)nutrient content per seagrass species. Elemental content of a) aluminium (Al), b) calcium (Ca), c) iron (Fe), d) potassium (K), e) magnesium (Mg), f) manganese (Mn), g) sodium (Na), h) sulphur (S), i) silicate (Si) and j) zinc (Zn) was determined as % of dry weight (DW) in samples from seagrass leaves. Straight dotted lines indicate the median element values of all samples and bold lines within boxplots indicate median element content per seagrass species. Unique sample numbers (counts) are indicated behind the boxes.

observed for Zosteraceae. This family showed strong positive correlations between leaf content for N and Fe ($r = 0.71$, $p < 0.001$), N and Mn ($r = 0.70$, $p < 0.001$), and N and Si ($r = 0.82$, $p < 0.001$) and a strong negative correlation between leaf content for N and Mg ($r = -0.85$, $p < 0.001$). For the other families, only weak correlations, if any, were observed.

In contrast, correlations between leaf P content and leaf Al, Ca, K, Na, Mg, and S content were more distinct ($|0.4| < r < |0.8|$; Fig. S6). When distinguishing between families, strong relationships ($r > |0.7|$) were found in Zosteraceae between leaf P and leaf Fe, Mg and Si content, in Hydrocharitaceae between leaf P and leaf K, Na, Mg, and S content, and in Cymodoceaceae between leaf P and leaf Fe, Mg, and S content, respectively.

4. Discussion

For seagrasses, knowledge on the function and ranges of (micro) nutrients are strongly limited. We therefore provided a global overview of seagrass leaf element content (Al, Ca, Fe, K, Mg, Mn, Na, S, Si, Zn) of 25 seagrass species from all four seagrass families. We showed here that leaf contents of almost all studied elements varied between families (Zosteraceae, Cymodoceaceae, Posidoniaceae and Hydrocharitaceae), between species and within species. The abundant elements, i.e. Ca, K, Mg, Na, S, were comparable between all four seagrass families and only relative small differences for Al, Fe, Mn and Zn were observed between families. This is in contrast to observed patterns in (micro)nutrients in terrestrial plants (Watanabe et al., 2007), which could be related to the

low diversity (limited number of species across only 4 families) of seagrasses. The high intraspecific variation may indicate that environmental concentrations drive leaf element content rather than differences in evolutionary history or in physiology between species. Thus, leaf element content seems mainly site specific, depending on local availability of elements, with some differences between successional stages. This is supported by the observed low variation in leaf element content of three seagrass species growing in a mixed meadow in Indonesia. Furthermore, in our analysis of *Z. noltii* across four sites we observed very low Si and Fe levels in Mauritania although this environment is very rich in both elements (e.g. Romero et al., 2008; Klicpera et al., 2013). A possible explanation for the low leaf Fe content in Mauritania may be the high pore-water sulphide concentrations that strongly reduce Fe availability (Holmer et al., 2005). On the other hand, the low leaf Si content of *Z. noltii* in this area suggests that leaf Si content is more likely to be determined by seagrass physiology rather than environmental conditions.

Seagrass leaves contained elements in the overall order Na > K > Ca > Mg > S > Fe > Al > Si > Mn > Zn. Compared to closely related submerged freshwater macrophytes (Potamogetonaceae, Hydrocharitaceae, Haloragaceae, Ceratophyllaceae and Characeae), seagrasses showed strikingly different relative leaf element values. The order of the average leaf element content of submerged macrophytes belonging to these freshwater taxa across 20 lakes in China was K > Ca > S > Na > Mg > Fe > Mn > Zn > Si (Xing et al., 2015). Na, Mg and Si are relatively more abundant compared to the other elements in seagrasses than in these submerged freshwater macrophytes.
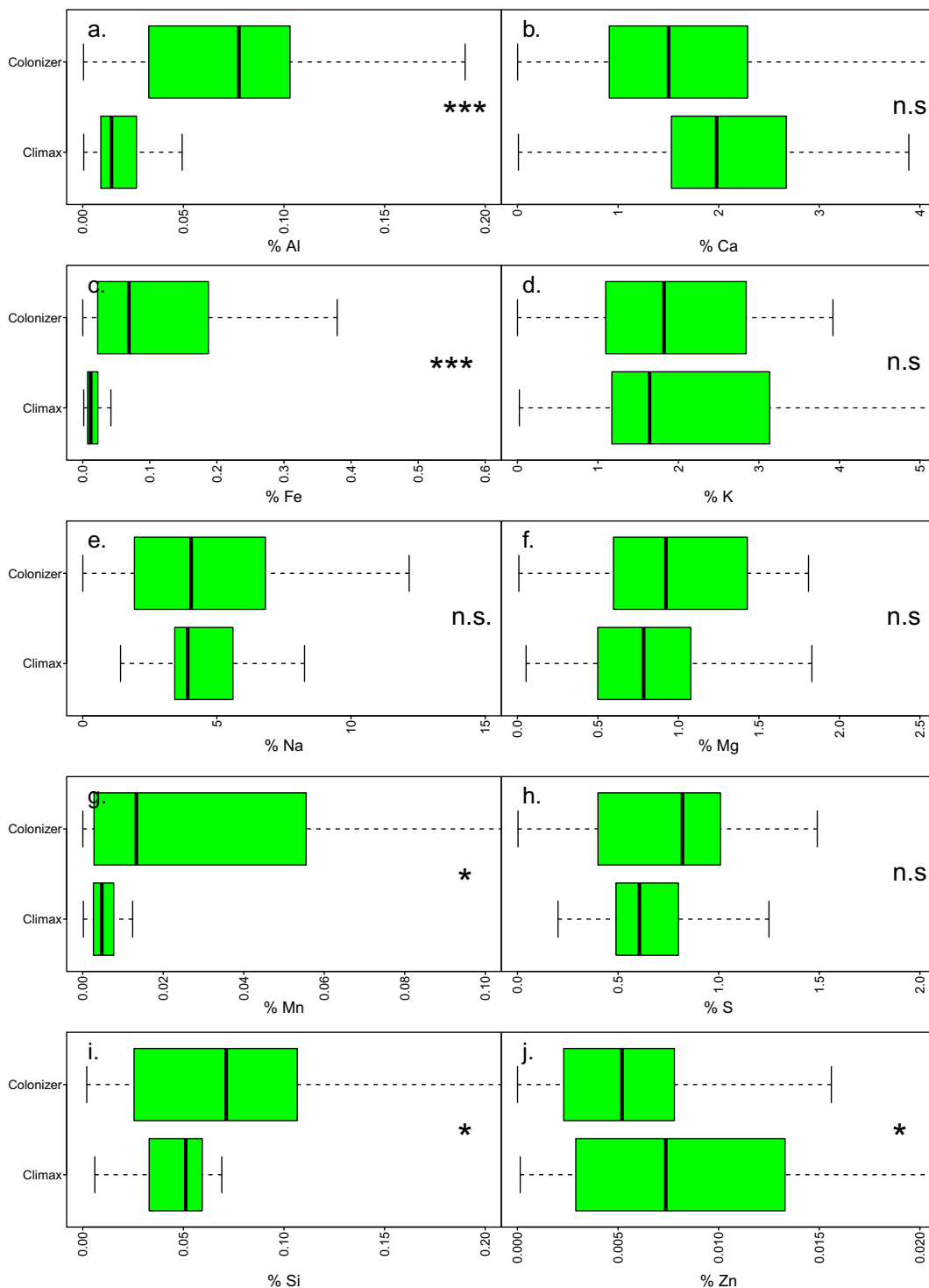
Fig. 4. Leaf (micro)nutrient content of early- and late-successional seagrass species for a) %Al (climax vs. colonizer, n = 41 vs. n = 55), b) %Ca (n = 25 vs. n = 49), c) %Fe (n = 77 vs. n = 104), d) %K (n = 26 vs. n = 55), e) %Mg (n = 28 vs. n = 46), f) %Mn (n = 60 vs. n = 85), g) %Na (n = 26 vs. n = 43), h) %S (n = 42 vs. n = 59), i) %Si (n = 42 vs. n = 39), and j) %Zn (n = 106 vs. n = 139), Stars indicate significance levels (*0.05 > p > 0.01, **0.01 > p > 0.001, ***p < 0.001) and n.s. indicates non-significant differences.

Differences in water chemistry between freshwater and marine systems, especially for dissolved Na and Mg, and large variation in water chemistry within freshwater ecosystems related to catchment geology, are strong drivers for altered leaf element content.

4.1. Seagrass leaf element content

The main driver for enhanced Na and Mg content in seagrasses is the higher salinity of the environment. Submerged freshwater macrophytes grow best under 3 psu salinity, and levels of > 18 psu are often toxic (Haller et al., 1974). Seagrasses are adapted to living in seawater with average optimal growth between 30 and 40 psu, but habitat conditions can range from as low as 9 psu (e.g. estuaries) to > 50 psu (e.g. mangrove lagoons) (Walker and McComb, 1990; Touchette, 2007). In order to account for osmotic adjustment and prevent dehydration, seagrasses increase their internal potential (Touchette, 2007). Na, accumulated in
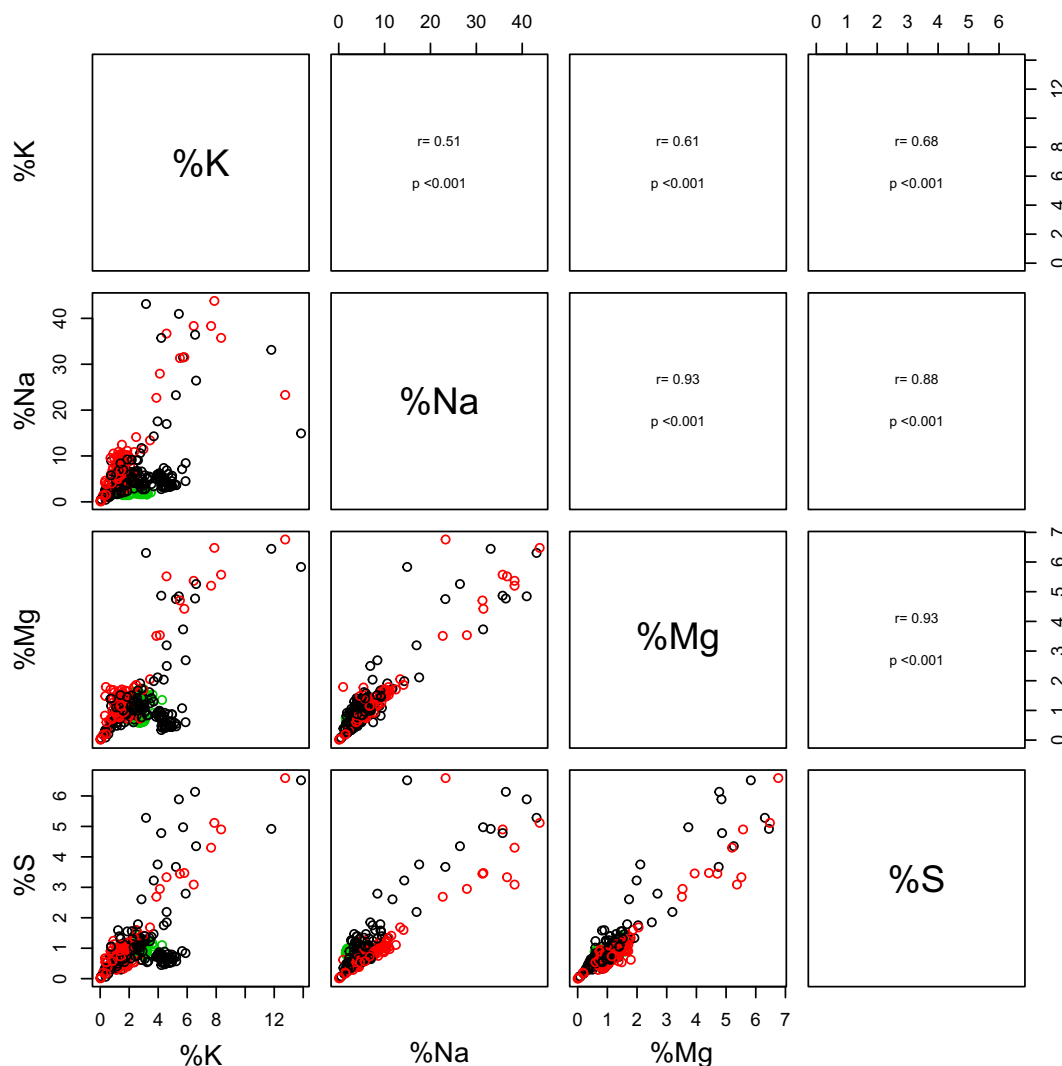
Fig. 5. Correlation matrix for leaf K, Na, Mg, and Si content, showing striking correlations between these leaf elements. Indicated values are in % DW and include only values of pairwise complete samples. The right upper panels indicate r- and *p*-values for each relationship. *p*-values lower than 0.05 are considered statistically significant. Colours indicate seagrass families: Cymodoceaceae in black, Hydrocharitaceae in red and Zosteraceae in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the leaves of seagrasses to maintain osmotic pressure, is probably largely stored in the vacuoles to minimize damage to cytosolic processes (Carpaneto et al., 1997). Observed Na content of seagrass leaves (0.003–22.35% DW) encompasses reported Na content of other halophytes like saltmarsh plants and mangroves (2.5–15.4% DW; Flowers et al., 1977; Subbarao et al., 2003).

Besides Na, seagrasses also contain high intracellular levels of K^+ compared to other angiosperms to overcome negative effects of osmotic stress. Olsen et al. (2016) reported genetic coding for the co-existence of proton transport and Na^+/K^+ antiporters in *Z. marina* likely to ensure maintenance of membrane potential against too high intrusion of Na from seawater. Large variation in molar Na:K ratios was observed between seagrasses, with the lowest values for temperate genera *Zostera* and *Amphibolis* (Na:K < 1.70) while for tropical species Na:K ratios up to 10 were observed. The average molar Na:K in seagrasses was 5.32 ± 0.44, about ten times less than the molar Na:K of surrounding seawater, highlighting the activity and importance of the Na exclusion mechanisms.

Magnesium is the central atom in chlorophyll and iron plays a major role in the electronic transport chain, with a molar Mg:Fe ratio of 40 required for adequate growth (Marschner, 2011). The relatively higher availability of Mg in seawater is reflected by an average Mg:Fe ratio of

59.5 ± 6.0 in seagrass leaves. The importance of Fe for productivity in seagrasses (Duarte et al., 1995) is reflected by the on average 5 × higher leaf Fe content of early successional compared to late successional seagrass species in our global database. Lower iron contents in the Posidoniaceae family compared to the other families may be explained by the fact that Posidoniaceae contains only late successional species whereas other groups contain both early and late successional species or that members of Posidoniaceae often grows in carbonate environments. Below a critical level of 100 μg Fe g DW^{-1} – or 0.01% DW Fe – it is a limiting component for seagrass growth (Duarte et al., 1995). The species with iron contents below the critical 0.01% DW limit in our database were all (tropical) species inhabiting carbonate sediments.

In seawater, sulphate (SO_4^{2-}) concentrations are about 100 × higher compared to fresh water (20–30 mM vs. 100–200 μM; Capone and Kiene, 1988). As salinity increases, methanogenesis is displaced by sulphate reduction as the primary pathway of carbon metabolism (Capone and Kiene, 1988). As a result of sulphate reduction, sulphide is produced which is a phytotoxin that may intrude roots and rhizomes of seagrasses during night time (Borum et al., 2005; Lamers et al., 2013). Although sulphide production depends on sediment characteristics (organic matter content and oxygen intrusion and consumption),

elemental sulphur is a major detoxification compound in seagrasses (Hasler-Sheetal and Holmer, 2015) and sulphide stress may result in elevated %S content of seagrasses (e.g. Van der Heide et al., 2012; Govers et al., 2014a; Hasler-Sheetal and Holmer, 2015). This is reflected by our results; we observed higher S content in seagrasses (0.77% DW) compared to freshwater species of the Hydrocharitaceae family (0.41% DW; Van der Welle et al., 2007). Finally, we also observed high correlations between leaf S content and elements related to osmotic stress (Na, K, and Mg) which could indicate the importance of sulphated polysaccharides in cell walls to facilitate water retention (Olsen et al., 2016).

High interspecific variation in Ca content can be attributed to presence or absence calcifying epiphytes or to internal calcium depositions. Brix and Lyngby (1984) concluded that Ca was the element which had the greatest concentrations in *Z. marina*, but also the greatest variation in values possibly related to the presence of non-removable calcareous epiphytic algae on the leaves. This has also been studied in other species that show high Ca values e.g. *T. testudinum*, *P. oceanica* and *C. nodosa* (Frankovich and Zieman, 1994; Martin et al., 2008; Shams El Din and El-Sherif, 2013). Secondly, a recent study discovered *T. testudinum* to precipitate aragonite calcium depositions internally in the cell wall and on the outside of the leaf, proving the direct contribution of seagrass to carbonate lime mud production (Enríquez and Schubert, 2014). It is still unknown in what quantities these crystals are produced whether the high concentration of Ca observed in other species can be explained by a similar mechanism, or if this phenomenon is exclusive to *T. testudinum*.

Beneficial effects of aluminium in the biochemical pathways of plants in general are yet unknown (Marschner, 2011). Although Al can enhance the growth and nutrient uptake of plants adapted to low pH soils (Osaki et al., 1997), in general Al^{3+} concentrations in the cytoplasm have been found to negatively impact photosynthetic electron transport and oxygen evolution in plants and some macroalgae (Thake et al., 2003; Delhaize et al., 2012). Variation in Al tolerance by plants is driven by the capacity to either exclude Al from their tissues or to detoxify Al once it enters the cytosol (Osaki et al., 1997; Delhaize et al., 2012) Seagrasses are probably not affected by direct Al toxicity, since they often have a cytoplasmatic pH above 6.0 at which Al in the form of $Al(OH)_4^-$ is harmless (Thake et al., 2003). For most metals, internal leaf content tends to increase in response to higher environmental concentrations of dissolved metals (Malea, 1993; Govers et al., 2014b). In the Mediterranean Sea, levels of Zn and Al are relatively high (Sherrell and Boyle, 1988; Chou and Wollast, 1997), and this is reflected in Zn levels found in *P. oceanica*, but not in Al levels of *P. oceanica* which were among the lowest observed for seagrasses. This is remarkable as *P. oceanica* is among the slowest growing seagrass species (Hemminga and Duarte, 2000) and this may indicate possible physiological adaptations of Posidoniaceae to limit Al uptake. Differences in leaf Al content were also observed between successional stages, with $4 \times$ higher values for early successional compared to late successional species. Although genetics of Al tolerance mechanisms have been investigated in a range of terrestrial species and underlying genes have been isolated (Delhaize et al., 2012), these are not yet studied in seagrasses.

Manganese and silicon are the least studied micronutrients in seagrasses. The importance of Mn for various redox processes in plant has been acknowledged (Jagtap, 1983; Marschner, 2011). In our study, Mn was the only element that showed no correlations with any other studied element in the leaves. Silicon is a beneficial micronutrient in higher plants in general. Silicon can increase the rigidity of plants in dynamic environments like streams and rivers (Schoelynck and Struyf, 2016) and might be important in stress situations like salt or metal stress (Liang et al., 2007; Wu et al., 2013). The average Si content of freshwater macrophyte, based on 83 different species, was 0.45% DW (Schoelynck and Struyf, 2016), around $5 \times$ higher compared to the average content in seagrasses (0.082% DW Si). The functional role of Si

in seagrass is not clear. Restricted Si availability due to impact of dams was linked to the decline in health of the seagrass population in a Dutch estuary (Herman et al., 1996). However, no correlations between leaf Si content and elements related to osmotic stress (Na, K, Mg) were observed in our global overview.

4.2. Global-scale benchmark and recommendations

We have here compiled a global review of, to our best knowledge, all available literature on seagrass leaf element content and added unpublished data of twelve species from twenty locations and nine countries. The two datasets were largely complementary, as literature data were not equally divided among regions, contained dominantly temperate species (*Z. marina*) and Mediterranean species (*P. oceanica* and *C. nodosa*) and rarely included data of multiple elements measured in one sample.

Since we reported averaged values for leaf content only, our database is not suitable for analysing changes in leaf element content across seasons, although differences between growth and dormant stages have been reported (Schlacher-Hoenlinger and Schlacher, 1998; Li and Huang, 2012). Seagrasses, like all rooted submerged macrophytes, can take up nutrients not only through the roots but also through the leaves (Romero et al., 2006). Govers et al. (2014b) showed that for many essential metals, leaf and root content were comparable. However, especially for elements involved in redox-processes, e.g. Fe, Mn, and S, exposure and uptake can be driven by sediment conditions, resulting in differences between above and belowground parts. Development of anoxic conditions due to mineralization, strongly related to sediment porosity, organic matter content and temperature, and the resulting increase in sulphide could impair uptake of nutrients like Fe (Lamers et al., 2013). Since the amount of data that is currently available on these topics is very sparse, it would be very interesting to further investigate the changes between seasons and differences between aboveground and belowground parts, especially in context of the importance of belowground seagrass biomass (Vonk et al., 2015).

Dynamics in and the role of various elements in the productivity of seagrasses should receive more attention (Van Tussenbroek et al., 2006). Elements considered as 'macro nutrients' in agriculture or terrestrial ecology (K, S, Ca, and Mg) are probably not limiting in the marine environment, given the high concentration at which they naturally occur (Romero et al., 2006). For many of the studied elements, leaf content showed correlations with the macronutrients N and especially P. This would indicate that productivity of seagrasses, reflected by P content, also (partly) depends on other nutrients or that fast growing plants can deal better with adverse conditions related to e.g. high Na content. Besides N and P, Fe is the only other element that has been independently confirmed to moderate seagrass productivity (Duarte et al., 1995).

To conclude, from our global overview consisting of 1126 unique leaf element values, we provide more insight into the role and importance of ten elements in seagrass physiology and functioning. Although we observed some differences in leaf element content between seagrass families, the high intraspecific variation is indicative that the leaf Al, Ca, Fe, K, Mg, Mn, Na, S, Si, and Zn content is more strongly determined by environmental factors than by evolutionary history. Higher leaf Al and Fe content of early successional species compared to late successional species indicate the importance of these elements for seagrass productivity. The elemental ratios were highly variable except for elements related to osmosis (Na, K, Mg) and S. Further analyses of the impacts of elements on productivity and adaptation are required. The soon expected genomes of other seagrass species (Olsen et al., 2016) in combination with experiments manipulating (micro)nutrients and environmental drivers (e.g. Garrote-Moreno et al., 2015) might enable us to unravel the importance of various elements to sustain productive and flourishing seagrass meadows.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2017.09.066.

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