plant biology

Plant Biology ISSN 1435-8603

RESEARCH PAPER

Internal and external regulation of plant organ stoichiometry

V. Minden & M. Kleyer

Landscape Ecology Group, Institute of Biology and Environmental Sciences, University of Oldenburg, Oldenburg, Germany

Keywords

Bivariate line-fitting; element ratio; growth rate hypothesis; heterostasis; homeostasis; standardised major axis analysis; tissue nutrient concentration.

Correspondence

V. Minden, Landscape Ecology Group, Institute of Biology and Environmental Sciences, University of Oldenburg, D-26126 Oldenburg, Germany.
E-mail: vanessa.minden@uni-oldenburg.de

Editor

D. Byers

Received: 24 July 2013; Accepted: 18 December 2013

doi:10.1111/plb.12155

ABSTRACT

Internal differences between plant organs are caused by the functional differentiation of plant tissue, whereas external supply rates of elements constrain nutrient uptake. Previous studies have concentrated on foliar or whole-plant stoichiometric response to the environment, whereas investigation of organ-specific comparisons is still pending. We explore C:N:P ratios of stems, leaves, diaspores and belowground organs in marsh plants, and evaluate the influence of environmental constraints using standardised major axis regression (SMA). For a pooled dataset, SMA resulted in distinct patterns of isometric and anisometric slopes between plant organs. Bivariate line-fitting for a split dataset of four ecological groups revealed that species of the frequently inundated marsh had higher N:C ratios than those of the infrequently inundated marsh. The influence of nutrient availability was detectable in decreased P:C and increased N:P ratios in P-poor sites. Across ecological groups, leaves and diaspores showed higher elemental homeostasis than stems and belowground organs. Any change in N:C ratios of belowground organs and diaspores in response to the environment was accompanied by an even stronger internal change in stem N:C ratios, indicating a pivotal role of stems of herbaceous plants in ecosystem processes. We found distinct patterns of C:N:P ratios in plant organs related to their internal function and external environmental constraints. Leaves and diaspores showed a higher degree of homeostasis than stems and belowground organs. We detected a clear external signal in element:element ratios of plant organs, with low soil P translating into lower tissue P:C ratio and stronger N retention in leaves as a response to salt stress.

INTRODUCTION

Photoautotrophs individually acquire elements (*e.g.* C, N and P) necessary for growth and maintenance, mostly as inorganic compounds, whereas heterotrophs mostly ingest elements bound into the biochemical structure of food (Ågren 2004). The latter may adjust their ingestion and assortment of food in order to sustain elemental homeostasis. Homeostasis refers to the maintenance of constant body concentrations despite fluctuations in environmental resources (Cannon 1929; Bradshaw *et al.* 2012). In contrast, heterotrophs, such as plants and algae, vary more strongly in their C:N:P ratios (Elser *et al.* 2000; Hessen *et al.* 2004). For instance, foliar N concentrations can vary across species by an order of magnitude within a single Amazonian rain forest site (Kraft *et al.* 2008), probably reflecting different functional niches in an ecosystem. Frost *et al.* (2005) provided examples for stoichiometric regulation of physiological processes in autotrophs, *e.g.* uptake of N in excess of metabolic demand or storage of elements in the vacuole.

Total amounts of nutrients, as well as the ratio of tissue concentrations, are crucial for the performance of a plant in its specific habitat. At an ecosystem level, tissue C, N and P content influences energy and material cycles (Elser *et al.* 2000), *e.g.* decomposability of litter (Hättenschwiler & Jorgensen 2010; Freschet *et al.* 2012), consumption of litter by herbivores (Jonas & Joern 2008) and C storage (Cebrian 1999).

Metabolic demand for particular elements is affected by supply in the environment and by biological stressors (Vrede *et al.* 2004; Zhang *et al.* 2012). This has led to a large body of literature relating the plants to the soil nutrient concentration to assess nutrient limitation (see reviews of Güsewell 2004; Ågren 2008). On the other hand, various studies have shown that plant stoichiometric ratios do not fully reflect variations in soil N:P ratios (Elser *et al.* 2010). In plants, relationships among size, biomass and function often depend on allocation to metabolically active or structural tissues and their stoichiometric composition. For instance, supporting structures, *e.g.* stems and branches, require the production of C-rich, low nutrient material, whereas fast-growing species invest in P-rich ribosomal RNA to rapidly convert photosynthetic products into growth (Elser *et al.* 2010). The growth rate hypothesis of Sterner & Elser (2002) states that increasing growth rates require more N and P relative to C and more P relative to N (Ågren 2008). This hypothesis is reflected in the leaf economics spectrum, a popular concept in plant functional ecology (Wright *et al.* 2004). According to this concept, a trait gradient from high to low leaf N content and high to low specific leaf area can be interpreted as a spectrum running from rapid growth, resource acquisition and turnover in leaves to slow growth and resource conservation. The latter is reflected in a decrease in biomass N:C content as a conservation strategy when nutrients are limiting (De Deyn *et al.* 2008).

So far, most studies relating plant stoichiometry to environmental conditions have focused only on whole plant or leaf stoichiometry (Wright *et al.* 2001; Güsewell & Koerselman 2002; Hessen *et al.* 2004). However, element composition differs strongly between plant organs (Ågren 2008). Tissue differentiation into roots, stems, leaves and seeds implies a specialisation of plant function, *e.g.* for both provision and storage of water and nutrients by roots, C gain by leaves, support for stems and regeneration through seeds. For example, leaves containing a high proportion of chloroplasts generally contain more N per unit dry mass than either stems or roots and have high N:P ratios (Epstein 1972). Flowering plant parts (shoots) may have a lower N:P ratio than non-flowering plant parts of the same species, and seeds generally have a lower N:P ratio and higher N and P concentration than vegetative structures (Fenner 1986; Güsewell 2004). To understand ecological stoichiometry in the framework of element allocation to plant functions such as growth, persistence and regeneration, it is necessary to compare the C:N:P ratio of each plant organ. The extent to which organ-specific stoichiometric patterns respond to environmental conditions or which plant organs exhibit stronger homeostasis, *i.e.* fixed elemental composition, in any environment to perform their functions is still an open question. For instance, communities growing in resource-poor environments could show lower N:C and P:C ratios in all organs than those of resource-rich environments, and this increase would be similar across all organs (see Vitousek 1982; Ågren 2008; Matzek & Vitousek 2009). Moreover, plants can also shift stoichiometry between organs in different environments. In this case, for example, a decrease in the N:C and P:C ratios of stems across a plant would be stronger than that of the leaves when the environment provides less resources. Here we address these questions by determining (i) general scaling relationships of element:element ratios between diaspores, stems, leaves, roots and rhizomes; and (ii) their responses to environmental conditions. We focus on C, N and P as the most important plant elements (Sterner & Elser 2002) and factor all plant organs considering their functional differentiation.

Our study was located in northwest European salt marshes, one of the few ecosystems in northwest Europe composed exclusively of herbaceous plant species (plus one shrub). As opposed to other terrestrial habitats, salinity and inundation frequency are major stress factors for plants inhabiting salt marshes (Rozema *et al.* 1985). To prevent inactivation of enzymes and other essential structures under high salinity, salt marsh species synthesise N-requiring osmoprotectants (Riadh *et al.* 2010). These 'compatible osmotic solutes' often constitute a large part of the plant N budget (Rozema *et al.* 1985), and we expect this to be reflected in the C:N:P ratios. Comparable to other terrestrial habitats, the availability of nutrient is crucial for species performance and biomass production in salt marshes (Ungar 1991). Nutrients such as K and P are highest in lower parts of a salt marsh and decrease with seaward distance (Hazelden & Boorman 1999). Salt marshes on clayey soils contain more available N than those on sandy soils (Olff *et al.* 1997).

To investigate the influence of the environment on the stoichiometry of plant organs, we distinguished four different ecological groups, *i.e.* species growing in (i) nutrient-rich, infrequently inundated habitats (RI); (ii) nutrient-rich, frequently inundated habitats (RF); (iii) nutrient-poor, infrequently inundated habitats (PI); and (iv) nutrient-poor, frequently inundated habitats (PF). Our specific expectations were:

1 C:N:P ratios would differ between plant organs according to their function. Metabolically active organs require more N and P relative to C than structural organs.
2 N:C would increase from low to high groundwater sites and infrequently to frequently inundated sites, together with N:P, due to synthesis of N-requiring osmoprotectants under increasing salinity (Bakker *et al.* 1993; Minden *et al.* 2012).
3 N:C and P:C ratios of leaves, stems and roots & rhizomes would increase from nutrient-poor to nutrient-rich environments, and N:P ratios would decrease, as growth rates increase with increasing available soil nutrients (growth rate hypothesis; see Sterner & Elser 2002; Ågren 2008; Matzek & Vitousek 2009).
4 Heterostasis would be indicated by shifts in response to the environment. For instance, N:P ratios of leaves and stems could show a parallel increase when sites are more frequently inundated, or leaf N:P ratios could increase more strongly than those of stems, which would indicate different degrees of heterostasis in plant organs.

MATERIAL AND METHODS

Study areas

Fieldwork was carried out in three study areas along the coastline of Lower Saxony, Germany, and on the island of Mellum. Despite a change in nutrient concentration along the elevation/inundation gradient, soils of the Wadden Sea islands generally provide fewer nutrients than those along the mainland coast. These so-called barrier islands initially developed from sand banks; the substrate drifted and continuously accumulated in response to sea currents, forming a chain of islands off the coast of northwest Germany. In contrast, the mainland marshes were created using land reclamation methods, in which parts of the Wadden Sea were gradually impoldered and drained (Pott 1995).

On the mainland, three study areas were surveyed: Leybucht (53°32′ N, 7°07′ E, eight plots), Norderland (53°40′ N, 7°19′ E, 89 plots) and Jade Bight (53°26′ N, 8°09′ E, 32 plots). The prevailing substrate is clayey silt, loamy sand and loamy silt. The island of Mellum (53°43′ N, 8°08′ E, 33 plots) originated from sand deposits transported by currents along the coast from The Netherlands and northwest Germany (Pott 1995), and now belongs to the core zone of the Wadden Sea National Park of Lower Saxony. The predominant substrate on the island is sand. Mean annual temperature is 9 °C and precipitation ranges from 770 to 830 mm $year^{-1}$ (west to east; German Meterological Service (DWD) 2013).

Within the study areas, distribution of plots was based on random stratified sampling, with elevation as stratification criterion (see Krebs 1989). All 162 plots were sampled during the growing season of 2007.

Environmental parameters

Soil fertility was determined by sampling each soil layer at each plot. Sampling depth was restricted to 30 cm due to high groundwater levels, which made identification of deeper layers

unfeasible. From the soil samples, the following parameters were investigated in the laboratory: bulk density (Schlichting *et al.* 1995), sand content as a proxy for N concentration (Olff *et al.* 1997; Ad-Hoc-AG Boden 2005), calcium carbonate (according to Scheibler, in Schlichting *et al.* 1995), plant available K (Flame photometer; Egnér *et al.* 1960) and P (Continuous Flow Analyser (CFA); Murphy & Riley 1962).

Lower salt marshes are flooded more regularly and for longer periods than upper marshes, which influences plot groundwater levels. A drainage pipe (6.5-cm diameter, 162 in total) was buried 80 cm vertically in the ground at each plot. In these pipes, the groundwater level was recorded twice weekly from May to September 2007 at low tide, as was salinity of the groundwater via conductivity measurement ('WTWpH/Cond 340i/SET').

Inundation frequencies and groundwater levels at high tide were determined with data loggers ('divers'; ecoTech). A total of 18 loggers documented the water column in selected pipes each hour over the period of measurement. For calculating the relative pressure of water accumulating in the pipe, four additional data loggers recorded pressure of the ambient air. Inundation frequency was calculated from the elevation of all plots relative to the water level measured by the data loggers.

Plant species and nutrient stoichiometry

Almost all abiotic variables differed strongly between mainland and island salt marshes. Soils of mainland plots were richer in nutrients than those of the island (compare environmental parameters in Table 2 and Fig. 2). Thus, mainland and island marshes were considered different habitats for this study, and mainland habitat types were termed 'Rich' (R) and island habitat types 'Poor' (P) in the split dataset. Consequently, we distinguished between populations of species from the mainland and the island (*e.g. Aster tripolium* from the mainland and *Aster tripolium* from the island; Table S1). Plant material was collected for 242 individuals in total, *i.e.* ca. 10 individuals per species. These individuals were selected from different plots covering the total range of species' occurrences within the environmental space.

Plants were collected at the peak of their generative stage, *i.e.* when seeds were ripe but had not yet been shed. Time of collection differed between species; some species had ripe seeds earlier than others and were thus harvested at an earlier time. We decided on this method based on the premise that allocation patterns change during ontogeny and can therefore be best compared when all species have reached the same phenological stage; however, we do not have data to support this premise. Plants were excavated, roots and rhizomes were cleaned of soil material by rinsing off the soil, and roots of different individuals were carefully separated using tweezers. For chemical analysis, plants were separated into leaves, stems, diaspores and belowground organs (hereafter referred to as 'roots & rhizomes'). Dead leaves were sorted and excluded from further analysis. For each organ of each individual, plant material was ground in a planetary mill at 300–400 revolutions ('pulverisette 7'; Fritsch, Idar-Oberstein, Germany). For C:N analysis, each sample was dried at 105 °C for 4–5 h. Then 2–3 mg of material was placed into tin tubes (0.1 mg precision balance CP 225 D; Sartorius, Göttingen, Germany) and analysed using a CHNS Analyser Flash EA (Thermo Electron Corp., Waltham, MA, USA) following Allen (1989). For measurement of P, 7–8 mg of material (dried at 70 °C) was processed with nitric acid and hydrogen peroxide and measured using a continuous flow analyser (CFA) following Murphy & Riley (1962). Some plant organs did not provide sufficient material for chemical analysis (*e.g.* roots & rhizomes of *Festuca rubra*), so data from such samples were omitted (Table S1).

Species composition and abundance were evaluated in each plot with frequency analysis using a 1 m × 1 m frame subdivided into 100 grid squares. Species nomenclature followed Jäger (2011).

Statistical analyses

Groundwater level

Groundwater level data lacked information on tidal variation, as biweekly field measurements were restricted to low tide; however, data loggers provided hourly recordings of groundwater level at 18 plots, comprising the entire groundwater level range from low to high tide. A regression was conducted with paired mean values from the hourly recordings and from the biweekly recordings at each individual plot. The regression function was then used to adjust mean groundwater level values of all plots to assess tidal variation across all plots.

Bivariate line-fitting methods

We used standardised major axis (SMA) slope-fitting techniques to explore bivariate relationships between the plant traits (*i.e.* element:element ratios of the various tissue types), as both the *X* and *Y* variables varied in measurement error, which made common linear regression inappropriate (Sokal & Rohlf 1994; Warton *et al.* 2006). The aim of the line-fitting technique is to summarise the relationship between two variables by minimising the residuals in both variables (Kerkhoff *et al.* 2006), rather than predict *Y* from *X*, for which ordinary least squares (OLS) regression would be an adequate technique (Niklas 2006).

When dividing the dataset according to the different environments, the clouds of points in an SMA diagram may show different alignments. First, two sites may show a shift along the common slope (site 1 and site 2, shift a, Fig. 1). If so, then both sites contain species sharing the same ratios between two traits, but species from one site generally have higher trait values (*i.e.* higher values on the *X*-axis with subsequently higher *Y*-values). Species from two sites showing a difference in one trait at a given second trait value (shift in *Y* combined with no change in *X*) show a shift in elevation among two lines (site 1 and site 3, shift b), and will have different ratios between the traits (see Warton *et al.* 2006). The method illustrates both general scaling relationships in organ-specific C:N:P ratios and their environmental response, which we consider as an advantage over multivariate techniques such as RLQ or GLMM used to assess trait–environment relationships (Jamil *et al.* 2012; Kleyer *et al.* 2012).

To test whether N:C, P:C and N:P ratios generally differ between plant organs, we first analysed each possible combination of element:element ratios of plant organs regardless of ecological group ('pooled dataset'). Second, to assign species to ecological groups occurring at different levels of inundation, salinity and soil nutrients, we conducted a constrained correspondence analysis (CCA) with environmental parameters and

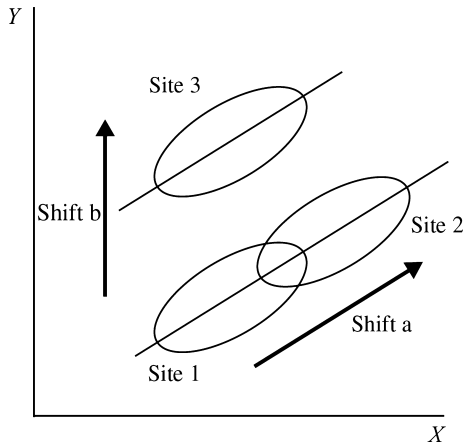

Fig. 1. Possible alignments of clouds of points sharing a common slope for traits *X* and *Y*, adapted from Wright *et al.* (2001). Site 1 and site 2 show a shift along a common slope (shift a), whereas site 1 and site 3 show a shift in elevation (shift b).

species abundance data. Species scores of the first two CCA axes were subjected to cluster analysis to group the species according to their position along the environmental gradients ('split dataset'). We then tested for significant correlations between each element:element ratio for pairs of plant organs of each species group at $P < 0.05$ (Pearson's r). The further analysis consisted of three steps and only considered relationships of the pair-wise N:C, P:C and N:P ratios of the plant organs where significant correlations existed. First, we tested for homogeneity of slopes with $P > 0.05$. Where a common slope could be fitted, we tested for differences in elevation and shift along the axis (steps 2 and 3; Wald statistic). Data on tissue nutrient content were log-transformed and analyses were performed using 'cca' (González *et al.* 2008), 'hclust' (Murtagh 1985; Ward's method) and 'sma' (Warton) for the computer software R (R Foundation for Statistical Computing 2012).

RESULTS

General stoichiometric relationships among plant organs

Across all plants, diaspores were most C-, N- and P-rich, which for N and P yielded significant differences in all other plant organs (Table 1, upper part, values in percentages). Nitrogen in stems was most variable, whereas C varied little.

Mean N:C and P:C ratios in stems and roots & rhizomes were lower than in leaves and diaspores (Table 1, upper part). N:P ratios of the four plant organs were more similar to each other, with the N:P ratio of roots & rhizomes being lowest and leaves being the highest. Across all species, heterostasis expressed as relative standard deviation was highest in stems, and (with one exception in P:C ratio) second highest in roots & rhizomes. Scaling relationships of N *versus* C (P *versus* C and

Table 1. *Upper*: Mean and relative standard deviation (RSD,%) of C, N and P content (mg g^{-1}; below for N:C, P:C and N:P ratios) for each plant organ across all ecological groups ('pooled dataset', no log transformation). Different letters indicate significant differences between plant organs (ANOVA, $P < 0.05$). *Middle:* N content of plant organs as a function of C (also P as a function of C and N as a function of P) across all ecological groups. Correlation coefficient, slopes of SMA regression (log-log) and 95% confidence intervals in parentheses. *Lower*: Statistics of SMA slopes (log-log) of each pair-wise combination between plant organs. Correlation coefficient, slope of the regression and 95% confidence interval in parentheses. Isometric slopes are shown in bold, NS indicates a non-significant relationship; all other relationships are significant at $P < 0.05$.

mean (RSD)	C	N	P
stem	417.4 (8.8)[a]	8.5 (52.3)[a]	1.0 (54.9)[a]
leaf	382.6 (12.6)[b]	17.7 (33.0)[b]	1.8 (41.6)[b]
diaspores	433.9 (10.9)[a]	22.5 (26.1)[c]	2.7 (39.1)[c]
roots & rhizomes	377.2 (19.5)[b]	8.5 (38.5)[a]	1.2 (50.8)[d]

mean (RSD)	N:C	P:C	N:P
stem	0.021 (53.8)[a]	0.002 (54.3)[a]	9.3 (48.6)[a]
leaf	0.048 (29.9)[b]	0.005 (43.5)[b]	10.0 (24.0)[a]
diaspores	0.053 (24.4)[b]	0.006 (39.8)[c]	9.6 (28.6)[a]
roots & rhizomes	0.023 (46.6)[a]	0.003 (42.7)[d]	6.7 (33.7)[b]

scaling relationships	N *versus* C *(Y versus X)*	P *versus* C *(Y versus X)*	N *versus* P *(Y versus X)*
stem	NS	NS	0.33, 0.80 (0.71, 0.92)
leaf	0.36, 2.53 (2.10, 2.90)	0.37, 3.18 (2.56, 3.73)	0.44, 0.70 (0.62, 0.81)
diaspores	0.27, 2.21 (1.61, 2.69)	NS	0.47, 0.65 (0.56, 0.74)
roots & rhizomes	0.45, 1.77 (1.19, 2.05)	0.27, 1.41 (0.79, 1.78)	0.38, 1.07 (0.87, 1.28)

Y	*X*	N:C	P:C	N:P
stem	leaf	0.41, 1.44 (1.23, 1.63)	0.40, 1.39 (1.15, 1.56)	0.41, 1.45 (1.26, 1.61)
stem	diaspores	0.49, 1.74 (1.49, 1.97)	0.50, 1.27 (1.11, 1.42)	0.57, 1.10 (0.74, 1.36)
stem	roots & rhizomes	0.48, **0.91 (0.75, 1.05)**	NS	0.33, **1.45 (1.25, 1.61)**
leaf	diaspores	0.56, **1.10 (0.90, 1.26)**	0.46, **0.92 (0.74, 1.09)**	0.41, **0.90 (0.75, 1.04)**
leaf	roots & rhizomes	0.37, 0.66 (0.55, 0.76)	0.22, 0.77 (0.53 2.09)	NS
diaspores	roots & rhizomes	0.35, 0.65 (0.53, 0.75)	NS	NS

N *versus* P) yielded anisometric slopes (<1; Table 1, middle part) for all plant organs except N *versus* P in roots & rhizomes. SMA regression of the pooled dataset was conducted to verify general C:N:P patterns between pairs of plant organs. Four relationships were not significant (Table 1, lower part). Linear increases of ratios of one organ with another organ (slope ~1) were found between stem and roots & rhizomes (both for N:C and N:P ratios) and between leaves and diaspores (for N:C, P:C and N:P ratios). All other relationships were anisometric (positive slope $\neq 1$), *i.e.* the increase of one organ was either stronger or less strong than that of the other organ considered. The increase in N:C, P:C and N:P ratios in stems was stronger than in either leaves or diaspores (slope > 1), whereas the increase in roots & rhizomes was less strong than in leaves and diaspores (slope < 1).

Environmental constraints on the stoichiometry of plant organs

The CCA analysis of environmental variables explained 24% of the inertia, of which 19% was explained through the first two axes (Fig. 2). Potassium, P, $CaCO_3$ and sand spanned a 'nutrient' gradient from nutrient-rich to nutrient-poor sites and also separated mainland from island plots. A second 'salt–waterlogging stress' gradient, uncorrelated to the first gradient, was determined in relation to inundation frequency, groundwater salinity and groundwater level. This separated highly inundated sites with high levels of salty groundwater from infrequently inundated sites with low groundwater levels.

Four clusters were positioned in the interspace between the major ordination gradients, *i.e.* cluster 1 contained species from infrequently inundated mainland sites with high nutrient content in the soil (RI; Fig. 2, Table 2 upper part), cluster 2 was positioned in nutrient-rich mainland sites with a high influence of salty groundwater and inundation (RF). Species of clusters 3 and 4 grew in nutrient-poor soils of the island and were positioned at opposite sides of the water gradient (cluster 3 in infrequently, cluster 4 in frequently inundated sites). The resulting ecological groups were: nutrient-rich, infrequently inundated (abbreviated RI) *versus* nutrient-rich, frequently inundated (RF, both mainland) *versus* nutrient-poor, infrequently inundated (PI) *versus* nutrient-poor, frequently inundated (PF, both island). Names of species in each cluster (ecological group) are given in Table S1.

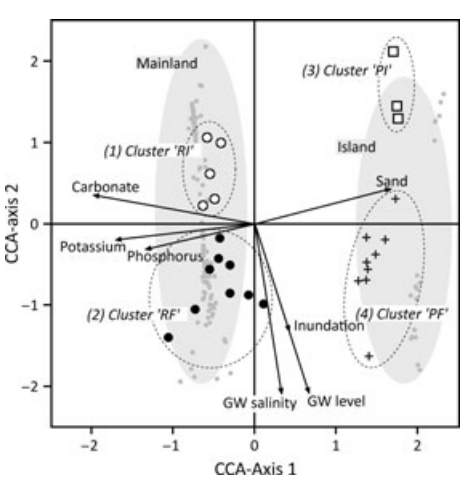

Fig. 2. Ordination diagram of the first two axes of CCA analysis displaying the environmental gradients and position of species (white and black circles, white squares and black crosses) with association to clusters (*i.e.* ecological groups) as a result of cluster analysis. Small grey dots represent plots. GW: groundwater. RI: nutrient-rich, infrequently inundated; RF: nutrient-rich, frequently inundated; PI: nutrient-poor, infrequently inundated; PF: nutrient-poor, frequently inundated.

Across all plant organs, N:C ratios were higher in frequently inundated habitats than in infrequently inundated sites with the same soil nutrient conditions (Table 2, Fig. 3, RF and PF *versus* RI and PI). N:P ratios showed similar patterns, whereas P:C ratios did not vary strongly between ecological groups. This indicates high N demand in response to inundation with salt water (see also mean C, N and P values in Table S1). Leaf N:P ratios of plants growing at infrequently inundated sites fell below the range for optimal growth (ratio < 10; see Güsewell 2004), indicating N limitation at these sites.

Bivariate line fitting resulted in 14 significant shifts along the slope and five shifts in elevation (Fig. 4, Table S2). Shifts along the slope were mainly detected for scaling relationships of stems with other organs, indicating parallel stoichiometric changes among organs in response to the environment. More specifically, species occurring in frequently inundated sites had significantly higher N:C ratios (Fig. 4a, c, e and f) in their particular organs than species in less frequently inundated sites with similar nutrient conditions. On the other hand, on sites with a similar water regime, organs of species growing under nutrient-rich conditions had significantly higher P:C and lower N:P ratios as compared to nutrient-poor conditions (Fig. 4h–l, n, o). This indicates that plant N responded mainly to inundation and salt whereas island plants were more P-limited than mainland plants, in response to lower soil P levels. Deviation from isometry also indicated stronger N retention in leaves and diaspores than in stems and roots & rhizomes.

Shifts in elevation were only significant when stems were related to diaspores or roots & rhizomes. These shifts indicate disproportionally stronger allocation of C relative to N to roots & rhizomes than to stems (and N relative to P to diaspores than to stems) when inundation frequency increases on poor sites (Fig. 4d and m). Conversely, stems receive disproportionally more C relative to P than diaspores (and less N relative to P than roots & rhizomes) when nutrients increase (Fig. 4i and n).

DISCUSSION

The C:N:P relationships between plant organs – internal regulation

The results clearly support our first expectation that C:N:P ratios differ between organs according to function (photosynthesis, supporting structure, storage, reproduction; see Wright *et al.* 2001; Kerkhoff *et al.* 2006). For instance, N:C, P:C and N:P ratios of stems were lower than those of leaves, indicating high C investment in supporting structures (Elser *et al.* 2010). Diaspores had a higher N:C ratio than leaves, as expected (Güsewell 2004), and N:P ratios were equal. Our results suggest that 'optimal' plant N:P ratios of 10–15 advocated in the literature (Linder 1995; Güsewell 2004) mainly apply to leaves, whereas other organs, roots in particular, have lower ratios.

Table 2. *Upper:* Mean and relative standard deviation (RSD,%) of environmental variables in each ecological group. Different letters indicate significant differences between ecological groups (ANOVA, $P < 0.05$). *Middle:* Mean and RSD of N:C, P:C and N:P ratios for each plant organ within each ecological group. *Lower:* Results of SMA for each combination of plant organs within each ecological group. Correlation coefficient, slope of the regression and 95% confidence interval in parentheses. Isometric slopes are shown in bold. NS indicates a non-significant relationship; all other relationships are significant at $P < 0.05$.

environmental variables mean (RSD,%)	nutrient-rich, infrequently inund.	nutrient-rich, frequently inund.	nutrient-poor, infrequently inund.	nutrient-poor, frequently inund.
phosphorus [kg ha^{-1}]	199.5 (28.0)[a]	190.3 (27.7)[a]	62.5 (29.3)[b]	125.5 (47.9)[c]
potassium [kg ha^{-1}]	1 796.6 (33.1)[a]	1 979.4 (24.7)[a]	696.4 (19.9)[b]	939.3 (39.8)[b]
$CaCO_3$ [t ha^{-1}]	210.4 (26.5)[a]	174.9 (37.2)[b]	14.6 (110.9)[c]	17.0 (157.8)[c]
sand content [%]	46.7 (44.2)[a]	43.6 (47.1)[a]	79.4 (11.3)[b]	65.1 (29.1)[b]
groundwater level [cm]	−38.2 (31.2)[a]	−23.1 (43.9)[a]	−36.2 (37.0)[a]	−17.4 (67.9)[b]
groundwater salinity [PSU]	21.3 (16.9)[a]	25.1 (13.7)[b]	21.4 (15.7)[a]	26.4 (5.6)[b]
inundation frequency [h]	38.4 (208.9)[a]	214.1 (136.0)[b]	169.2 (105.5)[a]	262.3 (140.1)[ab]

	nutrient-rich, infrequently inundated (RI)			nutrient-rich, frequently inundated (RF)		
element:element ratio mean (RSD,%)	N:C	P:C	N:P	N:C	P:C	N:P
stem	0.021 (80.0)	0.003 (58.6)	8.8 (88.2)	0.021 (41.3)	0.003 (46.4)	8.9 (45.2)
leaf	0.042 (30.4)	0.005 (49.9)	9.6 (38.4)	0.051 (22.9)	0.005 (30.4)	10.1 (29.8)
diaspore	0.051 (26.3)	0.006 (34.7)	8.7 (36.7)	0.056 (21.1)	0.007 (33.7)	8.9 (42.1)
roots & rhizomes	0.016 (36.3)	0.003 (74.7)	6.7 (39.8)	0.025 (44.7)	0.004 (35.4)	7.4 (30.8)

	nutrient-poor, infrequently inundated (PI)			nutrient-poor, frequently inundated (PF)		
element:element ratio mean (RSD)	N:C	P:C	N:P	N:C	P:C	N:P
stem	0.012 (36.8)	0.002 (61.5)	7.8 (66.8)	0.023 (38.6)	0.002 (57.6)	12.1 (44.9)
leaf	0.032 (24.9)	0.004 (30.0)	8.5 (25.8)	0.046 (33.5)	0.004 (36.2)	11.6 (31.8)
diaspores	0.037 (20.5)	0.004 (29.6)	9.7 (30.3)	0.056 (24.9)	0.006 (50.1)	11.1 (33.6)
roots & rhizomes	0.018 (42.5)	0.005 (50.9)	5.9 (40.6)	0.023 (45.4)	0.003 (43.5)	7.3 (38.7)

SMA of N:C		RI	RF	PI	PF
stem	leaf	0.64, 1.38 (0.77, 1.75)	0.36, 1.73 (1.27, 2.11)	NS	0.28, 1.18 (0.90, 1.48)
stem	diaspores	0.68, 2.12 (1.52, 2.63)	0.36, 1.88 (1.49, 2.23)	NS	NS
stem	roots & rhizomes	NS	0.49, **1.00 (0.75, 1.19)**	0.54, **0.96 (0.50, 1.29)**	0.37, 0.68 (0.48, 0.84)
leaf	diaspores	0.62, 1.51 (0.94, 1.91)	0.47, **0.99 (0.67, 1.21)**	NS	NS
leaf	roots & rhizomes	NS	NS	0.81, **1.05 (0.09, 1.34)**	0.31, 1.48 (1.01, 1.90)
diaspore	roots & rhizomes	NS	NS	NS	NS

SMA of P:C		RI	RF	PI	PF
stem	leaf	0.54, **1.17 (0.78, 1.47)**	0.33, 1.38 (0.98, 1.70)	NS	0.34, 1.51(1.14, 1.81)
stem	diaspores	0.52, 1.52 (1.02, 1.95)	0.36, 1.16 (0.90, 1.40)	0.52, 2.17 (1.31, 2.67)	**0.84, 1.12 (0.89, 1.33)**
stem	roots & rhizomes	NS	NS	NS	NS
leaf	diaspores	0.57, 1.32 (0.88, 1.70)	0.40, **0.84 (0.64, 1.03)**	NS	0.72, 0.55 (0.39, 0.72)
leaf	roots & rhizomes	NS	NS	NS	0.52, 0.84 (0.64, 1.0)
diaspore	roots & rhizomes	NS	−0.42, −1.02 (−1.22, −0.75)	NS	0.56, 1.21 (0.61, 1.54)

SMA of N:P		RI	RF	PI	PF
stem	leaf	NS	0.42, 1.52 (1.09, 1.84)	NS	0.43, 1.60 (1.03, 2.04)
stem	diaspores	NS	0.59, 1.24 (1.02, 1.42)	0.64, 1.91 (1.22, 2.54)	0.83, 1.46 (1.13, 1.80)
stem	roots & rhizomes	NS	0.47, 1.46 (1.09, 1.75)	NS	0.64, 1.16 (0.91, 1.35)
leaf	diaspores	0.42, 1.21 (0.71, 3.22)	0.35, 0.75 (0.57, 0.94)	NS	0.73, 0.71 (0.49, 0.87)
leaf	roots & rhizomes	NS	NS	NS	0.29, 0.78 (0.47, 1.0)
diaspore	roots & rhizomes	NS	0.43, 1.33 (1.0, 1.57)	NS	NS

Whether these differences can be generalised remains contentious. For instance, Knecht & Göransson (2004) report similar ratios for all organs across many ecosystems, whereas Kerkhoff *et al.* (2006) found lower values for roots than for leaves, where each paper used global datasets. However, most studies from natural environments only refer to foliar ratios and organ-specific comparisons require further attention.

We found distinct patterns of isometric and anisometric scaling relationships between organ-specific C:N:P ratios for the pooled dataset, *i.e.* across all species and sites. Isometric scaling characterised the C:N:P relationships between stems and roots & rhizomes as well as leaves and diaspores. Anisometric scaling was observed for all other significant combinations, *e.g.* stem *versus* leaf, stem *versus* diaspores, leaf *versus*

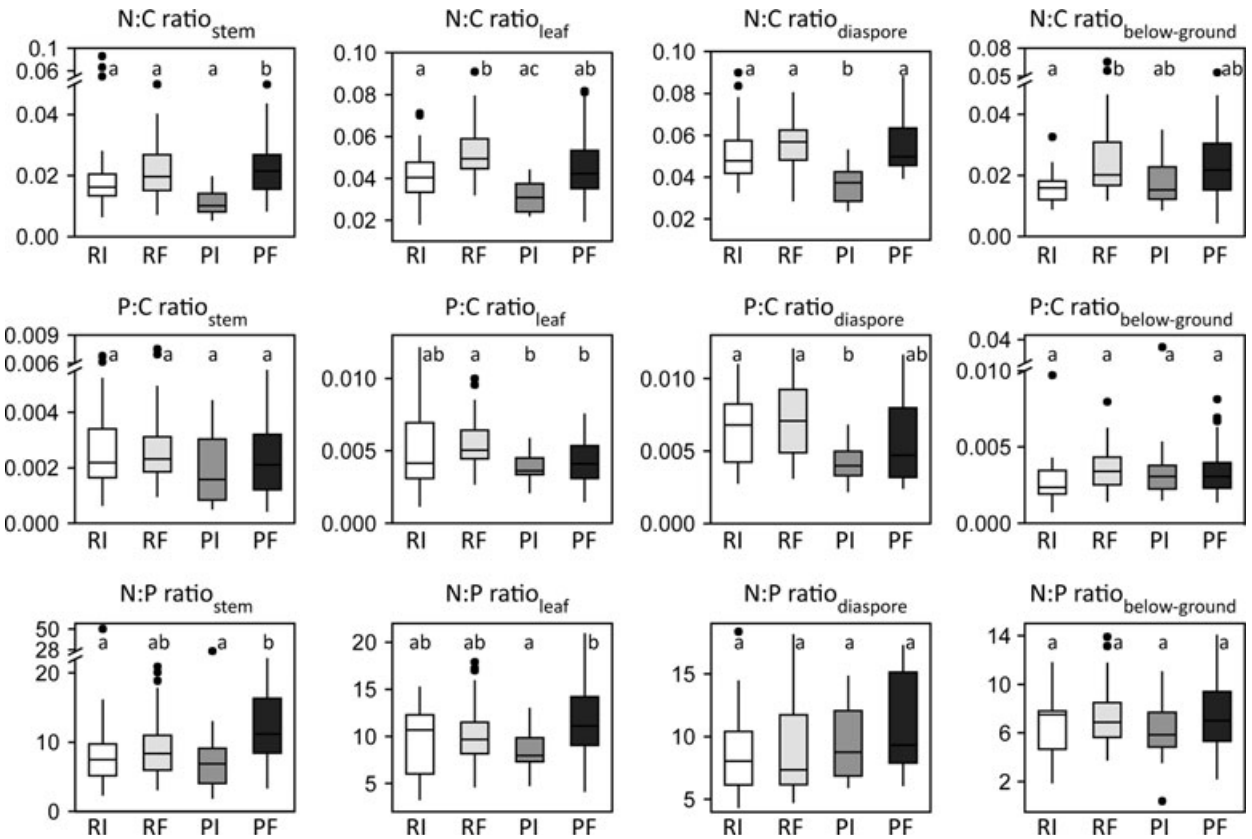

Fig. 3. Boxplots of N:C, P:C and N:P ratios of each plant organ for each ecological group. Significant differences (ANOVA, $P < 0.05$) between ecological groups are indicated by different letters in each box. RI: nutrient-rich, infrequently inundated; RF: nutrient-poor, frequently inundated; PI: nutrient-poor, infrequently inundated; PF: nutrient-poor, frequently inundated.

roots & rhizomes, etc. These results support the findings of Kerkhoff *et al.* (2006) who used a much larger dataset, spanning species from arctic to tropical regions, however without including environmental information. These authors focused on N and P content and applied the term 'structural' to group stems and roots & rhizomes and 'metabolic' to group leaves and diaspores, finding that stoichiometric scaling within these groups tended to yield isometric relationships whereas scaling between these groups was anisometric (either >1 for stem *versus* leaf and stem *versus* diaspores or <1 for leaf and diaspores *versus* roots & rhizomes). Our results fully support these findings and complement them insofar as the patterns also apply to N:C and P:C ratios.

Within the pooled dataset, the relationships between stem and leaf, and stem and diaspores scaled about 4/3-power for P:C, and about 4/3-power for N:C and N:P. Power law exponents for leaves *versus* roots & rhizomes, and diaspores *versus* roots & rhizomes, were about 2/3 to 3/4. In summary, stoichiometric change in one 'metabolic' organ (leaf or diaspore) implied the same change in the other metabolic organ. Conversely, changes in both 'metabolic' organs led to proportionally higher changes in stems and lower changes in roots & rhizomes. Similarly, Yu *et al.* (2011) found that stoichimetric regulation was inversely related between aboveground and belowground compartments. Consequently, stems appear to be most responsible for the variation in aboveground C and nutrient stocks whereas roots & rhizomes buffer these variations in the belowground ecosystem compartments.

The stoichiometric scaling can be compared to scaling of organ-specific biomass allocation in the same species set. We found isometric biomass scaling among leaves, stems and diaspores, whereas scaling exponents of leaf and diaspore biomass with roots & rhizome biomass were below 0.9 (Minden & Kleyer 2011). Niklas & Enquist (2002) found isometric scaling relationships between leaf and stem biomass, and leaf and root biomass. The discrepancy between biomass and stoichiometric scaling among plant organs shows that changes in vegetation C and nutrient stocks cannot be inferred from biomass changes alone.

The C:N:P relationships of plant organs in response to salinity and soil resources

In our study, salinity was lowest in the upper parts of the marsh and increased towards the lower parts where inundation is highest. Salinity represents a strong non-consumable environmental factor to which lower marsh halophytes respond by producing N-rich compatible osmotic solutes (Rozema *et al.* 1985; Rhodes *et al.* 2002; Flowers & Colmer 2008). Conversely, upper marsh species increase tissue lignin content as an adaptation to salt stress (Minden *et al.* 2012). Both adaptations should increase N:C and N:P ratios on lower salt marshes, particularly in leaves (expectation 2). Significant differences in N:C means and shifts along slopes confirmed our expectation (Figs 3 and 4). The responses to salinity were most pronounced on N-rich clayey soils, as indicated by low sand content. In

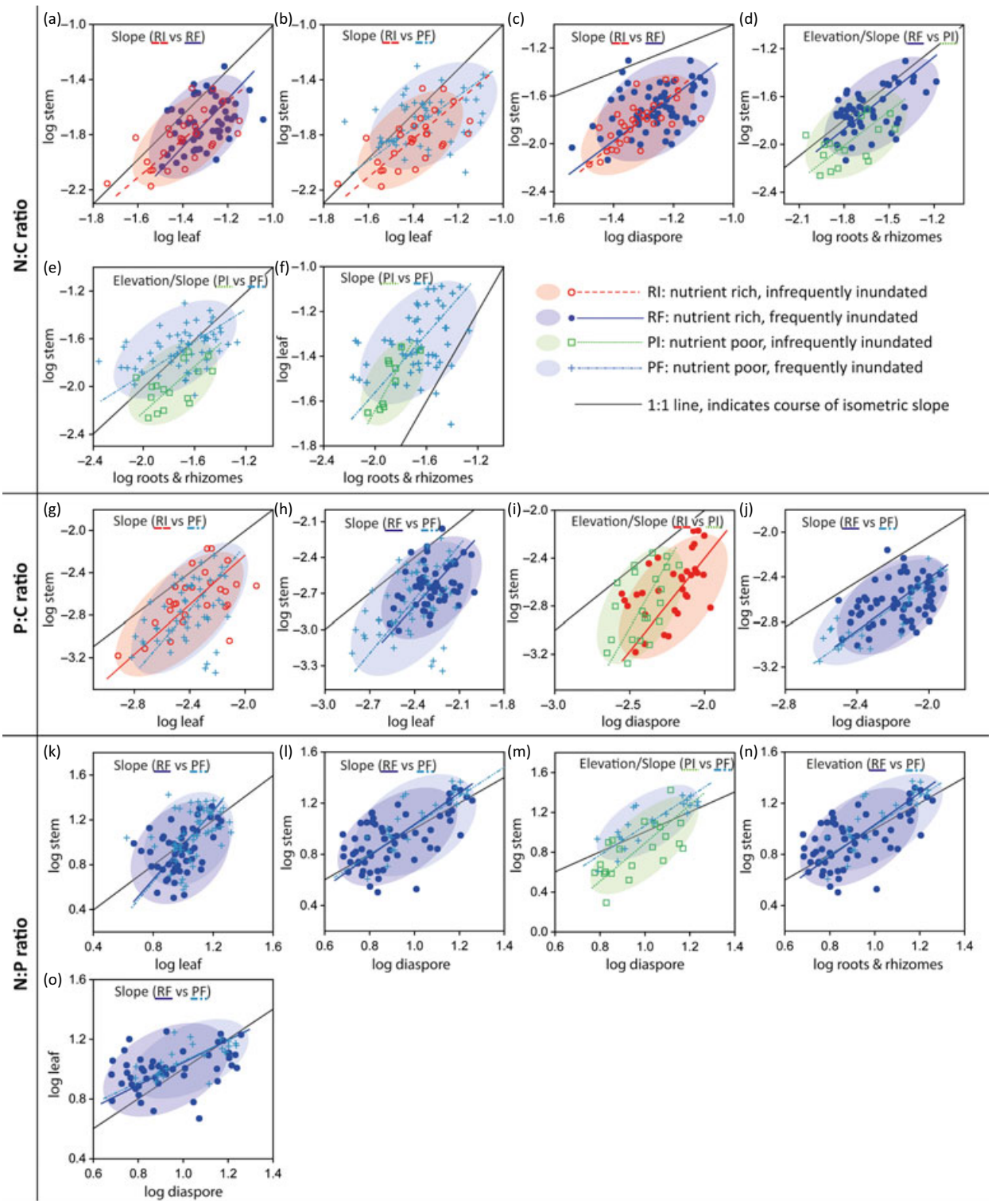

Fig. 4. SMA relationships of N:C, P:C and N:P ratios for combinations between stems, leaves, diaspores and roots & rhizomes for four ecological groups. Ecological groups inhabit sites that are (i) nutrient-rich, infrequently inundated (cluster RI: open red circle, dashed line), (ii) nutrient-rich, frequently inundated (cluster RF: closed dark blue circle, solid line), (iii) nutrient-poor, infrequently inundated (cluster PI: open green square, dotted line) and (iv) nutrient-poor, frequently inundated (cluster PF: light blue cross, dash–dot line). All relationships are significant at $P < 0.05$. Clouds around points aid in distinguishing between ecological groups. The 1:1 line is shown (grey) in each graph, indicating course of isometric slope. Slope: shift along slope, Elevation: shift in elevation.

Wadden Sea salt marsh soils, clay content is a reliable indicator for soil N supply (Olff *et al.* 1997).

We also assumed higher P:C and lower N:P ratios on more fertile soils where increasing growth rates should demand more P relative to N due to its functional role in the machinery of cell growth and metabolism (Sterner & Elser 2002; Ågren 2008). Indeed, both productivity and standing biomass were higher in P-rich sites compared to P-depleted habitats (data not shown). Moreover, P:C ratios increased with soil nutrients and this resulted in significant shifts along the slope (Fig. 4h–j). For N:P ratios, N accumulation in response to salinity and P accumulation to allow increased growth rates both intermingled when formulated as N:P ratio, which makes interpretation of whether or not patterns reflect higher growth rates in P-rich sites difficult. Bivariate line fitting showed significant shifts along the slope, which are associated with differences in nutrient regime between the sites (Fig. 4k, l, n and o), rather than a combination of responses to nutrient availability and salt stress. From this, we do believe that the N:P ratios of our study confirm the growth rate hypothesis to a certain extent; however more research is needed to disentangle the effects of N for osmoprotectants on growth rates and its subsequent reflection in tissue N:P ratios. Also, plant material was collected at the peak of generative time when seeds were ripe, thus assuming termination of the plant's life cycle for the sampling year. There is no information about investment in growth and/or allocation of elements to different organs beyond this time.

Shifts along the slope illustrate changes in element ratios in response to environmental conditions while allocation proportions are conserved, *i.e.* two or more organs experience the same change. In contrast, shifts in elevation indicate a disproportionate change in one organ compared to another. We only detected shifts in elevation when stems were compared to other organs. From the nutrient-poor, infrequently inundated sites (upper island marshes) to the nutrient-rich, frequently inundated sites (lower mainland marshes), N increased more strongly in stems than in roots & rhizomes and diaspores, either as a function of C or P. This suggests that the stoichiometry of stems is most responsive to environmental changes, whereas other organs achieve higher homeostasis. Kerkhoff *et al.* (2006) attributed stem C:N:P variations to a difference between woody and non-woody species. However, it also applies to the herbaceous plants of northwest European salt marshes. It appears that stems demand less osmoprotectants on upper marshes and invest more C in structural tissues as compared to the more 'metabolic' organs, whereas on lower marshes high N recycling in the phloem is necessary to allow the synthesis of more osmoprotectants.

Interestingly, C:N:P responses to environmental changes applied not only to leaves and stems, but also to diaspores. The amount of nutrients allocated to reproductive tissue plays a central role when characterising a plant 'ecological strategy'. Effects of nutrient supply on nutrient concentration in seeds may vary between plant species, ranging from keeping constant concentrations (Fenner 1986) to lower P concentrations in P-limited sites (Lewis & Koide 1990). It was also found that seed size is related to nutrient content, with larger seeds containing higher N and P concentrations than smaller seeds (Vaughton & Ramsey 2001; Obeso 2012).

Our analysis yielded limited significant shifts along the slope and with elevation, given the large number of possible combinations. Although we are certain our results mirror distinct patterns of plant responses to environmental fluctuations and not just random variability, more surveys are needed to confirm the results. Also, when considering salt as an environmental stressor in our study, data from non-halophytic communities are required to confirm the patterns found in the present study.

Organ-specific C:N:P homeostasis

Usually, stoichiometric homeostasis is seen as a species-specific property which is experimentally assessed by growing a species across a wide range of environmental N:P supply (*e.g.* Yu *et al.* 2011, 2012). Here we used the concept to compare interspecific stoichiometric responses to available soil P and sand content as an indicator of soil N, asking which plant organ shows stronger C:N:P regulation despite changes in environmental supply. It is widely accepted that environmental fluctuations are reflected in foliar stoichiometry, which is well suited to map factors such as edaphic controls on organ nutrient properties (Marschner 1995; Lambers *et al.* 2008; Stock & Verboom 2012). However, in our study leaves tended to exert higher homeostasis than stems, which were more responsive to environmental constraints and showed higher stoichiometric heterostasis. In their reviews Güsewell (2004) and Elser *et al.* (2010) reported H values as a measure of the degree of homeostasis in response to different resource supply rates for bacteria, algae and vascular plants (with $H = 1$ lack of homeostasis). Most studies indicate more or less strong homeostasis, but are either at the biomass level (Ryser & Lambers 1995) or for only one plant organ (see Ladanai *et al.* 2010; Persson *et al.* 2010 for algae; Yu *et al.* 2011). Although we cannot directly compare our results to the H values reported in various studies, we would expect leaves, diaspores and roots & rhizomes to show homeostasis, whereas H values for stems could be close to 1, *i.e.* close to or clear heterostasis.

In our study, shifts along the slope showed that C:N:P relationships among all organs responded to fertility and flooding frequency, indicating that salt marsh species do not maintain similar element ratios despite variations in element supply (Yu *et al.* 2011). Additionally, several indicators showed that stems were more responsive to environmental constraints and had higher heterostasis than other organs. First, scaling exponents of stems (y) *versus* other organs (x) were always >1, indicating a stronger change in stems. Second, only stems showed shifts in elevation. Third, relative standard deviations of stem N:P, P:C and N:P values were higher than for other organs in the pooled dataset and across all environmental regimes in the split data set. Together the results signal lower homeostatic regulation in this 'structural' organ than in the 'metabolic' organs.

CONCLUSIONS

The results of this study clearly demonstrate the existence of distinct patterns of C:N:P ratios in plant organs in relation to their function. We detected coordinated patterns of isometric and anisometric relationships between 'structural' (stem, roots & rhizomes) and 'metabolic' organs (leaf, diaspore) when investigating the pooled dataset. This suggests the existence of a set of common rules across many plants that govern the partitioning of nutrients among plant organs (Kerkhoff *et al.*

2006). Whether these rules depend on environmental conditions or on basic physiological requirements have rarely been investigated. Leaves and diaspores showed a higher degree of homeostasis in C:N:P ratio, indicating that C gain and reproductive functions are more constrained by basic physiological requirements than are structural support and nutrient uptake functions. However, we also detected an increased N demand in the lower marsh species, presumably due to enhanced production of N-demanding compatible osmotic solutes in order to avoid inactivation of enzymes and dehydration of the cytoplasm.

This is one of the first field studies that has investigated organ-specific stoichiometric responses to environmental conditions, where plant stems showed the strongest responses. These responses have significant effects on salt marsh ecosystem properties, such as decomposition rates and standing dead biomass (Minden & Kleyer 2011). Considering the wide range of C:N:P ratios in plants, and particularly in stems, changes in species occurrences along environmental gradients may therefore have profound effects on C and nutrient cycles. This may be obvious when forests are converted to arable fields, but our study however shows that these effects also apply to changes in natural herbaceous plant communities.

ACKNOWLEDGEMENTS

We thank the administration of the National park Niedersächsisches Wattenmeer and Mellumrat e.V. for support during fieldwork. Many thanks to H. Timmermann and J. Spalke for contributing their data, and to G. Scheiffarth and M. Heckroth for supporting our work at Mellum. We also thank Helmut Hillebrand for comments to an earlier version of the manuscript. This study was conducted as part of the TREIBSEL project, supported by the 'II. Oldenburgischer Deichband' and the 'Wasserverbandstag e.V.' (NWS 10/05).

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Names of species in each ecological group (RI, RF, PI and PF).

Table S2. Results of standardised major regression analysis for all significantly correlated trait combinations showing homogeneity of slopes for four ecological groups.

REFERENCES

Ad-Hoc-AG Boden (2005) *Bodenkundliche Kartieranleitung*. E. Schweitzerbart'sche Verlagsbuchhandlung, Hannover, Germany.

Ågren G. (2004) The C:N:P stoichiometry of autotrophs – theory and observations. *Ecology Letters*, **7**, 185–191.

Ågren G. (2008) Stoichiometry and nutrition of plant growth in natural communities. *Annual Review of Ecology, Evolution and Systematics*, **39**, 153–170.

Allen S.E. (1989) *Chemical analysis of ecological materials*. Blackwell Scientific, Oxford, UK, pp 368.

Bakker J.P., De Leeuw J., Dijkema K.S., Leendertse P.C., Prins H.H.T., Rozema J. (1993) Salt marshes along the coast of the Netherlands. *Hydrobiologia*, **265**, 73–95.

Bradshaw C., Kautsky U., Kumblad L. (2012) Ecological stoichiometry and multi-element transfer in a coastal ecosystem. *Ecosystems*, **15**, 591–603.

Cannon W.B. (1929) Organization for physiological homeostasis. *Physiological Reviews*, **9**, 399–431.

Cebrian J. (1999) Patterns in the fate of production in plant communities. *The American Naturalist*, **154**, 449–468.

De Deyn G.B., Cornelissen J.H.C., Bardgett R.D. (2008) Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology Letters*, **11**, 516–531.

Egnér H., Riehm H., Domingo W.R. (1960) Untersuchungen über die Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes des Bodens II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung. *Kungl. Lantbrukshögskolans Annualer*, **26**, 199–215.

Elser J.J., Fagan W.F., Denno R.F., Dobberfuhl D.R., Folarin A., Huberty A., Interlandi S., Kilham S.S., McCauley E., Schulz K.L., Siemann E.H., Sterner R.W. (2000) Nutritional constraints in terrestrial and freshwater food webs. *Nature*, **408**, 578–580.

Elser J.J., Fagan W.F., Kerkhoff A.J., Swenson N.G., Enquist B.J. (2010) Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytologist*, **186**, 593–608.

Epstein E. (1972) *Mineral nutrition of plants: principles and perspectives*. Wiley, New York, USA.

Fenner M. (1986) The allocation of minerals to seeds in *Senecio vulgaris* plants subjected to nutrient shortage. *Journal of Ecology*, **74**, 385–392.

Flowers T.J., Colmer T.D. (2008) Salinity tolerance in halophytes. *New Phytologist*, **179**, 945–963.

Freschet G.T., Aerts R., Cornelissen J.H.C. (2012) A plant economics spectrum of litter decomposability. *Functional Ecology*, **26**, 56–65.

Frost P.C., Evans-White M.A., Finkel Z.V., Jensen T.C., Matzek V. (2005) Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. *Oikos*, **109**, 18–28.

German Meterological Service (2013) Mean climate values for the period 1961 to 1990. Available from: http://www.dwd.de/bvbw/appmanager/bvbw/dwdwwwDesktop?_nfpb=true&_pageLabel=dwdwww_klima_umwelt_klimadaten_deutschland&T82002gsbDocumentPath=Navigation%2FOeffentlichkeit%2FKlima__Umwelt%2FKlimadaten%2Fkldaten__kostenfrei%2Fkldat__D__gebiete__rasterdaten__node.html%3F__nnn%3Dtrue (accessed 2 August 2013).

González I., Déjean S., Martin P.G.P., Baccini A. (2008) CCA: an R package to extend canonical correlation analysis. *Journal of Statistical Software*, **23**, 1–14.

Güsewell S. (2004) N: P ratios in terrestrial plants: variation and functional significance. *New Phytologist*, **164**, 243–266.

Güsewell S., Koerselman W. (2002) Variation in nitrogen and phosphorus concentrations of wetland plants. *Perspectives in Plant Ecology, Evolution and Systematics*, **5**, 37–61.

Hättenschwiler S., Jorgensen H.B. (2010) Carbon quality rather than stoichiometry controls litter decomposition in a tropical rain forest. *Journal of Ecology*, **98**, 754–763.

Hazelden J., Boorman L.A. (1999) The role of soil and vegetation processes in the control of organic and mineral fluxes in some western European salt marshes. *Journal of Coastal Research*, **15**, 15–31.

Hessen D.O., Agren G.I., Anderson T.R., Elser J.J., De Ruiter P.C. (2004) Carbon sequestration in ecosystems: the role of stoichiometry. *Ecology*, **85**, 1179–1192.

Jäger E.J. (2011) *Rothmaler Exkursionsflora von Deutschland Gefäßpflanzen: Grundband*. Spektrum Akademischer, Heidelberg, Germany.

Jamil T., Ozinga W.A., Kleyer M., ter Braak C.J.F. (2012) Selecting traits that explain species-environment relationships: a generalized linear mixed model approach. *Journal of Vegetation Science*, **24**, 988–1000.

Jonas J.L., Joern A. (2008) Host-plant quality alters grass/forb consumption by a mixed-feeding insect herbivore, *Melanoplus bivittatus* (Orthoptera: Acrididae). *Ecological Entomology*, **33**, 546–554.

Kerkhoff A.J., Fagan W.F., Elser J.J., Enquist B.J. (2006) Phylogenetic and growth form variation in the scaling of nitrogen and phosphorus in the seed plants. *The American Naturalist*, **168**, 103–122.

Kleyer M., Dray S., de Bello F., Leps J., Pakeman R.J., Strauss B., Thuiller W., Lavorel S. (2012) Assessing species and community functional responses to environmental gradients: which multivariate methods? *Journal of Vegetation Science*, **23**, 805–821.

Knecht M.F., Göransson A. (2004) Terrestrial plants require nutrients in similar proportions. *Tree Physiology*, **24**, 447–460.

Kraft N.J.B., Valencia R., Ackerly D.D. (2008) Functional traits and niche-based tree community assembly in an Amazonian forest. *Science*, **322**, 580–582.

Krebs C.J. (1989) *Ecological Methodology*. Harper & Row, New York, USA, pp 654.

Ladanai S., Ågren G.I., Olsson B.A. (2010) Relationships between tree and soil properties in *Picea abies* and *Pinus sylvestris* forests in Sweden. *Ecosystems*, **13**, 302–316.

Lambers H., Raven J.A., Shaver G.R., Smith S.E. (2008) Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology & Evolution*, **23**, 95–103.

Lewis J.D., Koide R.T. (1990) Phosphorus supply, mycorrhizal infection and plant offspring vigour. *Functional Ecology*, **4**, 695–702.

Linder S. (1995) Foliar analysis for detecting and correcting nutrient imbalances in Norway Spruce. *Ecological Bulletins*, **44**, 178–190.

Marschner H. (1995) *Mineral nutrition of higher plants*. Academic Press, London, UK.

Matzek V., Vitousek P.M. (2009) N: P stoichiometry and protein:RNA ratios in vascular plants: an evaluation of the growth-rate hypothesis. *Ecology Letters*, **12**, 765–771.

Minden V., Kleyer M. (2011) Testing the effect–response framework: key response and effect traits determining aboveground biomass of salt marshes. *Journal of Vegetation Science*, **22**, 387–401.

Minden V., Andratschke S., Spalke J., Timmermann H., Kleyer M. (2012) Plant–trait environment relationships in salt marshes: deviations from predictions by ecological concepts. *Perspectives in Plant Ecology, Evolution and Systematics*, **14**, 183–192.

Murphy J., Riley J.P. (1962) A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, **27**, 31–36.

Murtagh F. (1985) *Multidimensional clustering algorithms COMPSTAT lectures 4*. Physica, Würzburg, Germany.

Niklas K.J. (2006) A phyletic perspective on the allometry of plant biomass-partitioning patterns and functionally equivalent organ-categories. *New Phytologist*, **171**, 27–40.

Niklas K.J., Enquist B.J. (2002) On the vegetative biomass partitioning of seed plant leaves, stems, and roots. *The American Naturalist*, **159**, 482–497.

Obeso J.R. (2012) Mineral nutrient stoichiometric variability in *Hedera helix* (Araliaceae) seeds. *Annals of Botany*, **109**, 801–806.

Olff H., de Leeuw J., Bakker J.P., Platerink R.J., van Wijnen H.J., de Munck W. (1997) Vegetation succession and herbivory in a salt marsh: changes induced by sea level rise and silt deposition along an elevation gradient. *Journal of Ecology*, **85**, 799–814.

Persson J., Fink P., Goto A., Hood J.M., Jonas J., Kato S. (2010) To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. *Oikos*, **119**, 741–751.

Pott R. (1995) *Farbatlas Nordseeküste und Nordseeinseln*. Ulmer, Stuttgart, Germany, pp 288.

R Foundation for Statistical Computing (2012) *R version 2.15.1*. Vienna, Austria.

Rhodes D., Nadolska-Orczyk A., Rich P.J. (2002) Salinity, osmolytes, and compatible solutes. In: Läuchli A., Lüttge U. (Eds), *Salinity: environment–plant–molecules*. Kluwer, Dordrecht, the Netherlands, pp 181–204.

Riadh K., Wided M., Hans-Werner K., Chedly A. (2010) Responses of halophytes to environmental stresses with special emphasis to salinity. *Advances in Botanical Research*, **53**, 117–145.

Rozema J., Bijwaard P., Prast G., Broekman R. (1985) Ecophysiological adaptations of coastal halophytes from foredunes and salt marshes. *Vegetatio*, **62**, 499–521.

Ryser P., Lambers H. (1995) Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. *Plant and Soil*, **170**, 251–265.

Schlichting E., Blume H.P., Stahr K. (1995) *Bodenkundliches Praktikum*. Blackwell, Berlin, Germany.

Sokal R., Rohlf J. (1994) *Biometry: the principles and practice of statistics in biological research*. W.H. Freeman, New York, USA.

Sterner R.W., Elser J.J. (2002) *Ecological Stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, Princeton, NJ, USA, pp 439.

Stock W.D., Verboom G.A. (2012) Phylogenetic ecology of foliar N and P concentrations and N: P ratios across mediterranean-type ecosystems. *Global Ecology and Biogeography*, **21**, 1147–1156.

Ungar I.A. (1991) *Ecophysiology of vascular halophytes*. CRC Press, Boca Raton, FL, USA, pp 209.

Vaughton G., Ramsey M. (2001) Relationships between seed mass, seed nutrients, and seedling growth in *Banksia cunninghamii* (Proteaceae). *International Journal of Plant Sciences*, **162**, 599–606.

Vitousek P.M. (1982) Nutrient cycling and nutrient use efficiency. *American Naturalist*, **119**, 553–572.

Vrede T., Dobberfuhl D.R., Kooijman S., Elser J.J. (2004) Fundamental connections among organism C: N: P stoichiometry, macromolecular composition, and growth. *Ecology*, **85**, 1217–1229.

Warton D., Wright E.J., Falster D.S., Westoby M. (2006) Bivariate line-fitting methods for allometry. *Biological Reviews*, **81**, 259–291.

Wright I.J., Reich P.B., Westoby M. (2001) Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats. *Functional Ecology*, **15**, 423–434.

Wright I.J., Reich P.B., Westoby M., Ackerly D.D., Baruch Z., Bongers F., Cavenders-Bares J., Chapin T., Cornelissen J.H.C., Diemer M., Flexas J., Garnier E., Groom P.K., Gulias J., Hikosaka K., Lamont B.B., Lee T., Lee W., Lusk C., Midgley J.J., Navas M.-L., Niinements Ü., Oleksyn J., Osada N., Poorter H., Poot P., Prior L., Pyankow V.I., Roumet C., Thomas S.C., Tjoelker M.G., Veneklaas E.J., Villar R. (2004) The worldwide leaf economics spectrum. *Nature*, **428**, 821–827.

Yu Q.A., Elser J.J., He N.P., Wu H.H., Chen Q.S., Zhang G.M., Han X.G. (2011) Stoichiometric homeostasis of vascular plants in the Inner Mongolia grassland. *Oecologia*, **166**, 1–10.

Yu Q., Wu H., He N., Lu X., Wang Z., Elser J.J., Wu J., Han X. (2012) Testing the growth rate hypothesis in vascular plants with above- and below-ground biomass. *PLoS ONE*, **7**, e32162.

Zhang S.B., Zhang J.L., Slik J.W.F., Cao K.F. (2012) Leaf element concentrations of terrestrial plants across China are influenced by taxonomy and the environment. *Global Ecology and Biogeography*, **21**, 809–818.