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3 **Sodium hyperaccumulators in the Caryophyllales are characterised by both abnormally**  
4 **large shoot sodium concentrations and  $[\text{Na}]_{\text{shoot}} / [\text{Na}]_{\text{root}}$  quotients greater than unity**

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15 Running title: Sodium hyperaccumulation in Caryophyllales

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1 ABSTRACT

2

3 • **Background and Aims** Some Caryophyllales species accumulate abnormally large shoot  
4 sodium (Na) concentrations in nonsaline environments. It is not known whether this is a  
5 consequence of altered Na partitioning between roots and shoots. This paper tests the  
6 hypotheses (1) that Na concentrations in shoots ( $[Na]_{shoot}$ ) and in roots ( $[Na]_{root}$ ) are positively  
7 correlated among Caryophyllales, and (2) that shoot Na hyperaccumulation is correlated with  
8  $[Na]_{shoot} / [Na]_{root}$  quotients.

9 • **Methods** Fifty two genotypes, representing 45 Caryophyllales species and four species  
10 from other angiosperm orders, were grown hydroponically in a nonsaline, complete nutrient  
11 solution. Concentrations of Na in shoots and in roots were determined using inductively  
12 coupled plasma mass spectrometry (ICP-MS).

13 • **Key Results** Sodium concentrations in shoots and roots were not correlated among  
14 Caryophyllales species with normal  $[Na]_{shoot}$ , but were positively correlated among  
15 Caryophyllales species with abnormally large  $[Na]_{shoot}$ . In addition, Caryophyllales species  
16 with abnormally large  $[Na]_{shoot}$  had greater  $[Na]_{shoot} / [Na]_{root}$  than Caryophyllales species  
17 with normal  $[Na]_{shoot}$ .

18 • **Conclusions** Sodium hyperaccumulators in the Caryophyllales are characterised by  
19 abnormally large  $[Na]_{shoot}$ , a positive correlation between  $[Na]_{shoot}$  and  $[Na]_{root}$ , and  $[Na]_{shoot} /$   
20  $[Na]_{root}$  quotients greater than unity.

21

22 **Key words:** angiosperm, Caryophyllales, evolution, ionome, matK phylogeny, mineral  
23 composition, shoot and root partitioning, sodium (Na) hyperaccumulation

# 1 INTRODUCTION

2

3 Saline soils are defined as soils with an electrical conductivity (ECe) of at least 2 dS m<sup>-1</sup>  
4 (White *et al.*, 2017) to 4 dS m<sup>-1</sup> (Munns and Tester, 2008; Munns *et al.*, 2020), which is  
5 equivalent to 20 mM sodium chloride (NaCl) to 40 mM NaCl, respectively. Although saline  
6 soils are generally dominated by large concentrations of NaCl, they can have large  
7 concentrations of other mineral elements including calcium (Ca), magnesium (Mg) and sulfur  
8 (S) (Boon III and MacIntyre, 1968; del Carmen Martínez-Ballesta *et al.*, 2008; White *et al.*,  
9 2017). Saline soils are widespread in dry environments, and NaCl in the rhizosphere impairs  
10 the uptake of water by a plant by lowering the osmotic potential of the soil solution (Arora  
11 and Dagar, 2019). Once taken up by a plant via its roots, Na can inhibit the activity of  
12 enzymes in the cytoplasm and become toxic (Flowers *et al.*, 2015).

13 Halophytes are defined as plants that can complete their life cycle in the presence of large  
14 concentrations of NaCl in the rhizosphere, for example 80 mM NaCl (Santos *et al.*, 2016), or  
15 200 mM NaCl (Flowers *et al.*, 2015), or as plants that tolerate large concentrations of Na and  
16 Cl in their shoots (Flowers *et al.*, 2015). The growth of most crop species is inhibited when  
17 grown on soils with an ECe of 4 dS m<sup>-1</sup> or even less (Munns, 2005; Arora and Dagar, 2019),  
18 and less than ca. 0.25% of all angiosperm species can complete their life cycle when exposed  
19 to 200 mM NaCl (Flowers *et al.*, 2010).

20 Halophytism has evolved repeatedly in angiosperms (Flowers *et al.*, 2010; Bromham, 2015)  
21 and angiosperm species have developed various mechanisms for tolerating Na in the  
22 rhizosphere (Subbarao *et al.*, 2003; Munns, 2005). Species that take up Na readily and  
23 accumulate large concentrations of Na safely in their shoots ([Na]<sub>shoot</sub>), for example *Beta*  
24 *vulgaris* L. (Caryophyllales), have been termed “accumulators” (White *et al.*, 2017). Other  
25 species, for example *Triticum aestivum* L. (Poales), limit the uptake of Na from the

1 rhizosphere into roots and the subsequent transport of Na from roots to shoots. These species  
2 have been termed “excluders” (White *et al.*, 2017).

3 Although some species exhibiting C<sub>4</sub> photosynthesis require Na, it is not considered an  
4 essential mineral nutrient for plants in general (Broadley *et al.*, 2012), and Na deficiency does  
5 not occur in natural environments (Subbarao *et al.*, 2003; Pilon-Smits *et al.*, 2009). The  
6 compartmentalisation of Na and Cl into vacuoles enables plants to avoid toxic effects of large  
7 Na<sup>+</sup> and Cl<sup>-</sup> concentrations in plant tissues and lowers the osmotic potential of vacuoles. The  
8 lowered osmotic potential of vacuoles can be used for osmotic regulation by plants and can  
9 thus be beneficial to plants growing in saline or dry environments (Glenn and O’Leary, 1984;  
10 Flowers *et al.*, 2015; Munns *et al.*, 2020).

11 The Caryophyllales order contains the largest number of halophytic species (n = 74 species)  
12 of all angiosperm orders, totalling more than 21% of all known halophytes (Flowers *et al.*,  
13 2010). Many Caryophyllales, for example cacti, are adapted to saline or dry environments,  
14 and ancestors of extant Caryophyllales are thought to have evolved in dry mineral rich  
15 environments (Cuénoud *et al.*, 2002). Some Caryophyllales species have abnormally large  
16 [Na]<sub>shoot</sub> (> 4 mg g<sup>-1</sup> DW) when grown in nonsaline conditions (Broadley *et al.*, 2004; White  
17 *et al.*, 2017).

18 The trait of abnormally large [Na]<sub>shoot</sub> among 61 Caryophyllales species from ten families  
19 grown in the same nonsaline environment was defined by White *et al.* (2017) and the  
20 evolution of abnormally large [Na]<sub>shoot</sub> among these families has been explored previously.  
21 Recently, Ievinsh *et al.* (2021) defined Na hyperaccumulation for plant species growing in  
22 saline coastal habitats along the Baltic Sea. Although previously identified Caryophyllales  
23 species with abnormally large [Na]<sub>shoot</sub> (White *et al.*, 2017) also had the largest [Na]<sub>shoot</sub> in the  
24 study of Ievinsh *et al.* (2021), it appeared that the threshold for Na hyperaccumulation in  
25 shoots might differ between nonsaline and saline environments. The association between

1 abnormally large  $[Na]_{shoot}$  and the partitioning of Na between shoots and roots has not been  
2 examined. The following four hypotheses were tested in this study:

3 - Hypothesis 1: Caryophyllales species grown hydroponically in nonsaline solution can  
4 be attributed a “normal” or “abnormally large”  $[Na]_{shoot}$  phenotype as suggested by  
5 White *et al.* (2017).

6 - Hypothesis 2:  $[Na]_{shoot}$  is positively correlated with  $[Na]_{root}$  among Caryophyllales  
7 grown in the same environment.

8 - Hypothesis 3: The  $[Na]_{shoot} / [Na]_{root}$  quotient is correlated with shoot Na  
9 hyperaccumulation among Caryophyllales species grown hydroponically in nonsaline  
10 conditions.

11 - Hypothesis 4: Observations made for Caryophyllales grown hydroponically in  
12 nonsaline conditions can be generalised for Caryophyllales growing in other  
13 environments.

14

## 15 MATERIALS AND METHODS

16

### 17 *Experimental conditions*

18

19 A glasshouse experiment was conducted between September 2016 and January 2017 at The  
20 James Hutton Institute (UK; latitude 56°27'24.6"N, longitude 3°04'09.7"W). The experiment  
21 was performed on 52 angiosperm genotypes (Table 1) representing 45 Caryophyllales species  
22 and species representing four other angiosperm orders: *Brassica oleracea* L. (Brassicaceae;  
23 Brassicales), *Helianthus annuus* L. (Asteraceae; Asterales), *Hordeum vulgare* L. (Poaceae;  
24 Poales), and *Phlomis lychnitis* L. (Lamiaceae; Lamiales). The 45 Caryophyllales species

1 represented 42 genera and 13 families, and included four genotypes of *Beta vulgaris* L.  
2 (beetroot, chard, sea beet, sugar beet).

3 Seeds were sourced from commercial suppliers (Supplementary Data Table S1) and  
4 germinated on germination paper (Whatman, Little Chalfont, UK) soaked with deionised  
5 water in petri-dishes. The germination conditions (exposure to light, temperature) were  
6 chosen according to species requirements. Seedlings were transplanted to rockwool plugs  
7 (2.5 x 2.5 x 4 cm; Grodan, Hedehusene, Denmark) as soon as radicles were observed.  
8 Rockwool plugs were placed in plastic trays in the glasshouse in which the experiment was  
9 conducted and irrigated with tap water containing 0.14 mM Na. Rockwool plugs with  
10 established seedlings were transferred into a nutrient film technique (NFT) hydroponic  
11 system, similar to the one described by Broadley *et al.* (2003), three to five days after the  
12 germination of seeds. The recirculating nutrient solution contained 2 mM  $\text{Ca}(\text{NO}_3)_2$ , 2 mM  
13  $\text{NH}_4\text{NO}_3$ , 0.75 mM  $\text{MgSO}_4$ , 0.5 mM KOH, 0.25 mM  $\text{KH}_2\text{PO}_4$ , 0.1 mM FeNaEDTA, 30  $\mu\text{M}$   
14  $\text{H}_3\text{BO}_3$ , 25  $\mu\text{M}$   $\text{CaCl}_2$ , 10  $\mu\text{M}$   $\text{MnSO}_4$ , 3  $\mu\text{M}$   $\text{CuSO}_4$ , 1  $\mu\text{M}$   $\text{ZnSO}_4$  and 0.5  $\mu\text{M}$   $\text{Na}_2\text{MoO}_4$  and  
15 was replaced regularly according to plant growth rates. The pH of the nutrient solution was  
16 adjusted daily to pH 6 – pH 7 using 0.5 M KOH or 0.5 M  $\text{H}_2\text{SO}_4$ .

17 The hydroponic system comprised two groups of four flat bottomed gullies (10 cm width, 4.5  
18 cm height, 6 m length, angle ca.  $1^\circ$ ) made of polyvinyl chloride (PVC). Gullies of the same  
19 group were spaced 8.5 cm apart, with 40 cm space between the two groups of gullies. A fine  
20 fleece mesh was placed at the bottom of each gully to create an even nutrient film. Flat PVC  
21 strips were mounted on top of each gully. For each gully, 90 circular holes of 3.5 cm diameter  
22 were cut into the PVC strips, 3 cm apart, to hold the rockwool plugs. The holes were covered  
23 with small PVC strips when not occupied by a rockwool plug. The recirculating nutrient  
24 solution was held in two 200 L tanks, one for each group of four gullies, and pumped evenly  
25 into the gullies. The glasshouse was set to maintain 22°C day and 18°C night temperatures

1 with a day length of 16 hours using automatic venting, supplementary heating and additional  
2 lighting as described by White *et al.* (2017).

3 The experimental design was a randomised block design with each gully representing two  
4 blocks. Up to eight individual plants per genotype were grown in each set of four gullies. One  
5 replicate per gully x block combination was achieved. More replicates of genotypes that grew  
6 slowly, and for which additional seedlings were available, were grown in the hydroponic  
7 system. All plants were harvested during their vegetative growth phase. Plants were harvested  
8 15-84 days after transfer to the hydroponic system, depending on growth rates  
9 (Supplementary Data Table S1). Four species: (1) *Beta vulgaris* (Amaranthaceae;  
10 Caryophyllales), (2) *Helianthus annuus* (Asteraceae; Asterales), (3) *Hordeum vulgare*  
11 (Poaceae; Poales) and (4) *Sagina subulata* (Sw.) C.Presl (Caryophyllaceae; Caryophyllales)  
12 were grown in gullies that were supplied by both tanks. Harvested plants were rinsed in  
13 deionised water and separated into shoots and roots. Shoots and roots were dried separately in  
14 paper bags at 70°C for a minimum of 72 hours to achieve a constant dry weight (DW).  
15 Samples were milled to a fine powder using a ceramic ball mill (Retsch MM 200 or Retsch  
16 MM 301; Retsch, Haan, Germany) and accurately weighed powdered subsamples (c. 50 mg  
17 DW) were digested in nitric acid in closed vessels using a microwave digester (MARS  
18 Xpress, CEM Microwave Technology, Buckingham, UK) as described by White *et al.*  
19 (2012). Sodium concentrations in digested samples were measured using inductively coupled  
20 plasma mass spectrometry (ICP-MS; ELAN DRc; PerkinElmer, Waltham, USA) as  
21 described by White *et al.* (2012). An externally certified reference material (1573a tomato  
22 leaf standard; National Institute of Standards and Technology, NIST, USA) was included as  
23 an internal control. Multiple replicates of individual genotypes were combined for ICP-MS  
24 analyses if insufficient dried sample was available. There was insufficient root dry matter of  
25 *Melandrium keiskei* (Miq.) Ohwi (Caryophyllaceae; Caryophyllales) to determine its sodium

1 concentration. For this reason, data from *Melandrium keiskei* were excluded from the results  
2 described below.

3 Eighteen Caryophyllales species, representing six Caryophyllales families, were grown in  
4 both the hydroponic experiment performed here and experiments described by White *et al.*  
5 (2017). These species were *Agrostemma githago* L., *Amaranthus caudatus* L., *Amaranthus*  
6 *cruentus* L., *Armeria maritima* (Mill.) Willd., *Atriplex hortensis* L., *Beta vulgaris*,  
7 *Carpobrotus edulis* (L.) N.E.Br., *Cerastium tomentosum* L., *Delosperma cooperi* (Hook.f.)  
8 L.Bolus, *Dorotheanthus bellidiformis* (Burm.f.) N.E.Br., *Limonium sinuatum* (L.) Mill.,  
9 *Persicaria capitata* (Buch.-Ham. ex D.Don) H.Gross, *Phytolacca americana* L., *Plumbago*  
10 *auriculata* Lam., *Psylliostachys suworowi* (Regel) Roshkova, *Rheum palmatum* L., *Sagina*  
11 *subulata* and *Silene armeria* L. (Table 1).

12

### 13 *Data analysis*

14

15 Data analyses were conducted using R 3.4.3 (R Core Team, 2017) using the packages ape 5.0  
16 (Paradis *et al.*, 2004), ggplot2 3.2.1 (Wickham, 2016), phangorn 2.3.1 (Schliep, 2011),  
17 phytools 0.6-44 (Revell, 2012) and rentrez 1.1.0 (Winter, 2017). Shoot sodium concentrations  
18 ( $[Na]_{shoot}$ ) are expressed on a DW basis and variation is expressed as standard deviation (SD)  
19 of n observations unless indicated otherwise.

20 Block effects and differences between the two groups of four gullies supplied by each tank  
21 were tested by analysis of variance (ANOVA) using the “aov” function from base R (R Core  
22 Team, 2017) and a linear model of the form  $\log_e([Na]_{shoot}) \sim \text{tank} + \text{block} + \text{genotype} * \text{organ}$ .  
23 The tilde separates the response variable (left) from the explanatory variables (right) and the  
24 “\*” indicates a genotype x organ (i.e. shoot or root) interaction. The three genotypes barley  
25 (*Hordeum vulgare*; Poales), beetroot (*Beta vulgaris*; Caryophyllales) and sunflower

1 (*Helianthus annuus*; Asterales), that were grown in both groups of four gullies, were included  
2 in this analysis. The fourth species grown in both groups of four gullies, *Sagina subulata*  
3 (Caryophyllales), was not included in this analysis as multiple shoot and root samples had to  
4 be combined to obtain enough material for ICP-MS analyses.

5 The trait of abnormally large  $[\text{Na}]_{\text{shoot}}$  was defined by fitting log-normal distributions to the  
6 observed frequency distributions of  $[\text{Na}]_{\text{shoot}}$  using the function “rnorm” from base R. Log-  
7 normal distributions were compared to each other by conducting t-tests using the “t.test”  
8 function from base R. Species with marginal  $[\text{Na}]_{\text{shoot}}$  to either of the log-normal distributions  
9 were assigned to a distribution using the “pnorm” function from base R. Pearson’s linear  
10 correlation coefficients and significance tests for correlations between  $[\text{Na}]_{\text{shoot}}$  and root Na  
11 concentrations ( $[\text{Na}]_{\text{root}}$ ) were conducted using the “cor.test” function from base R. The  
12 “density” function from base R was used for visualising the empirical distributions of  
13  $[\text{Na}]_{\text{shoot}}$ ,  $[\text{Na}]_{\text{root}}$  and  $[\text{Na}]_{\text{shoot}} / [\text{Na}]_{\text{root}}$  of Caryophyllales species grown in the experiment  
14 (Fig. 1) based on kernel density estimates (KDE).

15 The mean  $[\text{Na}]_{\text{shoot}}$ ,  $[\text{Na}]_{\text{root}}$  and  $[\text{Na}]_{\text{shoot}} / [\text{Na}]_{\text{root}}$  of Caryophyllales families represented in  
16 the experiment were mapped to the matK phylogeny using the “contMap” function of the  
17 phytools package (Revell, 2012).

18 To construct the matK phylogeny of the thirteen Caryophyllales families represented in the  
19 experiment, amino acid sequences of the plastid gene *matK* were sourced using the NCBI  
20 protein database (Supplementary Data Table S2). Complete matK sequences were used for all  
21 genera, if possible, and partial matK sequences were sourced if no complete matK sequences  
22 were available. All amino acid sequences were obtained using the “entrez\_fetch” function  
23 from the rentrez package (Winter, 2017). All complete matK sequences were then aligned  
24 using MUSCLE 3.8.31 (Edgar, 2004). Unique partial matK sequences were then aligned  
25 iteratively against the alignment of complete matK sequences. A phylogenetic tree based on

1 maximum likelihood (ML) was inferred using the alignment of both complete and partial  
2 sequences using the package phangorn (Schliep, 2011). The BIC criterion (Bayesian  
3 Information Criterion), obtained using the “modelTest” function of phangorn, suggested a  
4 JTT + G + I model. The topology of the phylogenetic tree was optimised using nearest  
5 neighbour interchanges (NNI). The non-Caryophyllales species *Brassica oleracea*  
6 (Brassicales), *Helianthus annuus* (Asterales), *Hordeum vulgare* (Poales) and *Phlomis*  
7 *elliptica* Benth. (Lamiales) were used as an outgroup and were subsequently removed from  
8 the rooted tree of Caryophyllales families. Partial sequences of matK from each genus  
9 clustered together, as did the sequences of matK for all genera within a Caryophyllales  
10 family.

11 Local regression analyses of the data sourced from the literature (Supplementary Data Table  
12 S3) were based on locally estimated scatterplot smoothing (LOESS) using the package  
13 ggplot2 (Wickham, 2016). The association between the accumulation of Na and the elements  
14 chlorine (Cl), S, nitrogen (N), phosphorous (P), potassium (K), Mg and Ca in shoots of  
15 Caryophyllales was tested by fitting linear models of the form  $\log_e([\text{Element}]_{\text{shoot}}) \sim \text{type}$  for  
16 each element using the “lm” function from base R (R Core Team, 2017). The tilde separates  
17 the response variable (left) from the explanatory variable (right). The data for this analysis  
18 were sourced from supporting information Table S1 of Neugebauer *et al.* (2018).  
19 Caryophyllales species for which the element concentrations were determined were grouped  
20 into species with abnormally large Na concentrations (type “hyper”) and those with normal  
21 Na concentrations (type “normal”), respectively.

22

## 23 RESULTS

24

25 *Sodium (Na) concentrations in shoots and roots of angiosperms*

1

2 Sodium (Na) concentrations in shoots ( $[\text{Na}]_{\text{shoot}}$ ) and roots ( $[\text{Na}]_{\text{root}}$ ) were determined in 44  
3 Caryophyllales species and four non-Caryophyllales species (Supplementary Data Table S1).  
4 No significant ( $P < 0.05$ ) block effects or differences between the two groups of four gullies  
5 were observed for tissue Na concentrations of the three species present in both groups of four  
6 gullies, but differences in  $[\text{Na}]_{\text{shoot}}$  among genotypes ( $P < 0.001$ ), genotype x organ  
7 interactions ( $P < 0.001$ ) and differences between roots and shoots ( $P < 0.01$ ) were significant  
8 (Supplementary Data Table S4).

9 The  $[\text{Na}]_{\text{shoots}}$  of all four non-Caryophyllales species (*Brassica oleracea*, *Helianthus annuus*,  
10 *Hordeum vulgare* and *Phlomis lychnitis*) were smaller than the mean  $[\text{Na}]_{\text{shoot}}$  of the  
11 Caryophyllales species ( $1.550 \pm 3.418 \text{ mg g}^{-1} \text{ DW}$ ,  $n = 44$  species; Supplementary Data Table  
12 S1) and consistent with the rank order of  $[\text{Na}]_{\text{shoot}}$  of these species determined previously  
13 (White *et al.*, 2017). The  $[\text{Na}]_{\text{shoot}}$  of all four non-Caryophyllales species fell within the range  
14 of  $[\text{Na}]_{\text{shoot}}$  of Caryophyllales species that did not hyperaccumulate Na. The  $[\text{Na}]_{\text{root}}$  of  
15 *Helianthus annuus*, *Hordeum vulgare* and *Phlomis lychnitis* were also smaller than the mean  
16  $[\text{Na}]_{\text{root}}$  of the Caryophyllales species studied ( $0.854 \pm 0.856 \text{ mg g}^{-1} \text{ DW}$ ,  $n = 44$  species), but  
17 the  $[\text{Na}]_{\text{root}}$  of *Brassica oleracea* was larger than the mean  $[\text{Na}]_{\text{root}}$  of all Caryophyllales  
18 species. Nevertheless, the  $[\text{Na}]_{\text{root}}$  of all non-Caryophyllales species fell within the range of  
19  $[\text{Na}]_{\text{root}}$  of the Caryophyllales species.

20

21 *Shoot Na concentrations of Caryophyllales species*

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23 The  $[\text{Na}]_{\text{shoot}}$  of Caryophyllales species (Fig. 1) ranged from  $0.04 \pm 0.01 \text{ mg g}^{-1} \text{ DW}$  ( $n = 2$   
24 plants) in *Simmondsia chinensis* (Link) C.K. Schneid. (Simmondsiaceae) to  $14.09 \pm 1.2 \text{ mg g}^{-1}$   
25  $[\text{Na}]_{\text{shoot}}$  ( $n = 8$  plants) in *Carpobrotus edulis* (Aizoaceae). The distribution of  $[\text{Na}]_{\text{shoot}}$  of 44

1 Caryophyllales species did not appear to fit a single normal distribution, nor a small set of  
2 normal distributions (Fig. **1A,B**). However, the data did appear to fit a small number,  
3 possibly two, log-normal distributions (Fig. **1C,D**). The first log-normal distribution, which  
4 had an estimated mean  $[\text{Na}]_{\text{shoot}}$  of  $-0.975$  ( $\log_{10}$  mg g<sup>-1</sup> DW) with a standard deviation of  
5  $0.225$  ( $\log_{10}$  mg g<sup>-1</sup> DW), contained the 34 Caryophyllales species with the smallest  $[\text{Na}]_{\text{shoot}}$ .  
6 The second log-normal distribution, which had an estimated mean of  $0.682$  ( $\log_{10}$  mg g<sup>-1</sup>  
7 DW) and a standard deviation of  $0.368$  ( $\log_{10}$  mg g<sup>-1</sup> DW), contained the ten Caryophyllales  
8 species with the largest  $[\text{Na}]_{\text{shoot}}$ . The two distributions differed significantly ( $P < 0.001$ ),  
9 suggesting two phenotypes. Species with  $[\text{Na}]_{\text{shoot}}$  larger than ca.  $0.8$  mg g<sup>-1</sup> DW ( $\log_{10} = -$   
10  $0.1$ ) were more likely to belong to the second distribution and this threshold was used to  
11 assign Caryophyllales species a particular phenotype. The species with  $[\text{Na}]_{\text{shoot}}$  closest to  $0.8$   
12 mg g<sup>-1</sup> DW were attributed a particular phenotype by testing the probabilities of these species  
13 belonging to either the first or the second distribution. *Psylliostachys suworowi*  
14 (Plumbaginaceae;  $1.37 \pm 0.58$  mg g<sup>-1</sup> DW,  $n = 8$  plants) was attributed a phenotype of  
15 abnormally large  $[\text{Na}]_{\text{shoot}}$  ( $P = 0.069$ ) rather than a normal  $[\text{Na}]_{\text{shoot}}$  ( $P < 0.001$ ). *Cistanthe*  
16 *grandiflora* (Lindl.) Schltdl. (Portulacaceae;  $0.32 \pm 0.07$  mg g<sup>-1</sup> DW,  $n = 8$  plants) was  
17 attributed a normal  $[\text{Na}]_{\text{shoot}}$  ( $P = 0.016$ ) rather than an abnormally large  $[\text{Na}]_{\text{shoot}}$  ( $P < 0.001$ ).

18

#### 19 *Root Na concentrations of Caryophyllales species*

20

21 The  $[\text{Na}]_{\text{root}}$  among Caryophyllales species ranged from  $0.12 \pm 0.0001$  mg g<sup>-1</sup> DW ( $n = 2$   
22 plants) in *Simmondsia chinensis* (Simmondsiaceae) to  $4.90 \pm 0.7$  mg g<sup>-1</sup> DW ( $n = 8$  plants) in  
23 *Carpobrotus edulis* (Aizoaceae). The distribution of  $[\text{Na}]_{\text{root}}$  did not appear to fit a single  
24 normal distribution (Fig. **1A,B**) or a single log-normal distribution (Fig. **1C,D**). The  
25 distribution of  $[\text{Na}]_{\text{root}}$  resembled a highly skewed normal distribution (Fig. **1A,B**) or two

1 overlapping log-normal distributions (Fig. **1C,D**). The latter did not coincide with the two  
2 log-normal distributions observed for  $[Na]_{shoot}$  (Fig. **1C,D**). Overall, there was little  
3 correlation between  $[Na]_{root}$  and  $[Na]_{shoot}$  among Caryophyllales species (Fig. **1A,C**).  
4 However,  $[Na]_{root}$  and  $[Na]_{shoot}$  were significantly correlated among the ten species with  
5 abnormally large  $[Na]_{shoot}$  ( $r = 0.83$ ,  $P = 0.003$ ; Fig. **2B**), but were completely uncorrelated  
6 among the 34 species with normal  $[Na]_{shoot}$  ( $r = -0.001$ ,  $P = 0.996$ ; Fig. **2C**). The variation of  
7  $[Na]_{shoot}$  of the latter 34 species was relatively narrow ( $0.121 \pm 0.068$  mg g<sup>-1</sup> DW, n = 34  
8 species), whereas their  $[Na]_{root}$  ( $0.638 \pm 0.447$ ) varied widely (Fig. **2C**).

9

#### 10 $[Na]_{shoot} / [Na]_{root}$ of Caryophyllales species

11

12 Quotients of  $[Na]_{shoot} / [Na]_{root}$  ranged from  $0.038 \pm 0.0216$  (n = 2 plants) in *Talinum*  
13 *paniculatum* (Jacq.) Gaertn. (Talinaceae) to  $12.094 \pm 2.551$  (n = 7 plants) in *Atriplex halimus*  
14 L. (Amaranthaceae). The empirical distribution of  $[Na]_{shoot} / [Na]_{root}$  from 44 Caryophyllales  
15 species did not fit a single normal distribution (Fig. **1B**) or a single log-normal distribution  
16 (Fig. **1D**).

17 Caryophyllales species with abnormally large  $[Na]_{shoot}$  generally had  $[Na]_{shoot} / [Na]_{root}$   
18 quotients greater than unity, and Caryophyllales species with normal  $[Na]_{shoot}$  generally had  
19  $[Na]_{shoot} / [Na]_{root}$  quotients less than unity (Fig. **1B,D**). *Psylliostachys suworowi* ( $[Na]_{shoot} =$   
20  $1.375 \pm 0.580$ ,  $[Na]_{shoot} / [Na]_{root} = 0.848 \pm 0.346$ , n = 8 plants) was the only species with an  
21 abnormally large  $[Na]_{shoot}$  and a  $[Na]_{shoot} / [Na]_{root}$  quotient less than unity. The two  
22 Polygonaceae species *Emex australis* Steinh. ( $[Na]_{shoot} = 0.207 \pm 0.064$ ,  $[Na]_{shoot} / [Na]_{root} =$   
23  $1.263 \pm 0.470$ , n = 3 plants) and *Eriogonum arborescens* Greene ( $[Na]_{shoot} = 0.166 \pm 0.018$ ,  
24  $[Na]_{shoot} / [Na]_{root} = 1.380 \pm 0.817$ , n = 6 plants) did not accumulate abnormally large  
25  $[Na]_{shoot}$ , but had mean  $[Na]_{shoot} / [Na]_{root}$  quotients greater than unity. However, the  $[Na]_{shoot} /$

1 [Na]<sub>root</sub> quotients of these three species did not differ significantly ( $P < 0.05$ ) from unity.  
2 Thus, the designation of abnormally large [Na]<sub>shoot</sub> coincided with a [Na]<sub>shoot</sub> / [Na]<sub>root</sub>  
3 threshold of unity.  
4 Some species in the three Caryophyllales families Aizoaceae, Amaranthaceae and  
5 Plumbaginaceae had abnormally large [Na]<sub>shoot</sub> (Fig. **2A,B**), but no species in the remaining  
6 10 Caryophyllales families had abnormally large [Na]<sub>shoot</sub> (Supplementary Data Table S1).  
7 Some species from all of the 13 Caryophyllales families studied had normal [Na]<sub>shoot</sub> (Fig.  
8 **2C**). Five of the seven Aizoaceae species studied had abnormally large [Na]<sub>shoot</sub> and these  
9 species generally had large [Na]<sub>root</sub> also. Four of the seven Amaranthaceae species studied  
10 had abnormally large [Na]<sub>shoot</sub>: *Beta vulgaris* (all four genotypes: sugar beet, beetroot, sea  
11 beet, swiss chard), both *Atriplex* species (*A. halimus*, *A. hortensis*) and *Salicornia europaea*  
12 L.. The [Na]<sub>root</sub> of these four Amaranthaceae species were within the range of [Na]<sub>root</sub> of  
13 Caryophyllales species exhibiting normal [Na]<sub>shoot</sub> (Fig. **2A**). Thus, Amaranthaceae species  
14 with abnormally large [Na]<sub>shoot</sub> did not cluster together with Aizoaceae species exhibiting  
15 abnormally large [Na]<sub>shoot</sub> when [Na]<sub>shoot</sub> was plotted against [Na]<sub>root</sub> (Fig. **2B**). One of the  
16 four Plumbaginaceae species studied, *Psylliostachys suworowi*, had an abnormally large  
17 [Na]<sub>shoot</sub>. *Psylliostachys suworowi* also had a large [Na]<sub>root</sub>. The three *Amaranthus* species (*A.*  
18 *caudatus*, *A. cruentus*, *A. tricolor*; Amaranthaceae) with normal [Na]<sub>shoot</sub> had similar [Na]<sub>shoot</sub>  
19 / [Na]<sub>root</sub> quotients (Fig. **2C**).

20

21 *[Na]<sub>shoot</sub>, [Na]<sub>root</sub> and [Na]<sub>shoot</sub> / [Na]<sub>root</sub> quotient of Caryophyllales families*

22

23 The mean [Na]<sub>shoot</sub>, [Na]<sub>root</sub> and [Na]<sub>shoot</sub> / [Na]<sub>root</sub> quotient were mapped to a matK phylogeny  
24 of the Caryophyllales families represented in this study (Fig. **3**). Three of 13 Caryophyllales  
25 families studied had species with abnormally large [Na]<sub>shoot</sub>, namely the Aizoaceae,

1 Amaranthaceae and Plumbaginaceae (Supplementary Data Table S1). The mean  $[\text{Na}]_{\text{shoot}}$  was  
2 also largest in these families (Fig. 3A). The Aizoaceae also had the largest mean  $[\text{Na}]_{\text{root}}$  (Fig.  
3 3B). However, although the Amaranthaceae and Plumbaginaceae had large mean  $[\text{Na}]_{\text{shoot}}$   
4 (Fig. 3A), they did not have large mean  $[\text{Na}]_{\text{root}}$  (Fig. 3B), and Tallinaceae had small  $[\text{Na}]_{\text{shoot}}$   
5 but large  $[\text{Na}]_{\text{root}}$  (Fig. 3B). Thus, the phylogenetic relationships among Caryophyllales  
6 families differed for  $[\text{Na}]_{\text{shoot}}$  and  $[\text{Na}]_{\text{root}}$ . This is consistent with a lack of correlation  
7 between  $[\text{Na}]_{\text{shoot}}$  and  $[\text{Na}]_{\text{root}}$  among Caryophyllales species in general (Fig. 2C). The  
8 Aizoaceae and Amaranthaceae, the families with the most species with abnormally large  
9  $[\text{Na}]_{\text{shoot}}$  studied here, had the largest  $[\text{Na}]_{\text{shoot}} / [\text{Na}]_{\text{root}}$  quotients of all the Caryophyllales  
10 families (Fig. 3A). The Aizoaceae and Amaranthaceae were the only families with a mean  
11  $[\text{Na}]_{\text{shoot}}$  larger than  $1 \text{ mg g}^{-1} \text{ DW}$  and a mean  $[\text{Na}]_{\text{shoot}} / [\text{Na}]_{\text{root}}$  quotient greater than unity  
12 (Fig. 3A).

13

## 14 DISCUSSION

15

### 16 *Sodium (Na) concentrations in shoots and roots of angiosperms*

17

18 The mean shoot sodium concentration ( $[\text{Na}]_{\text{shoot}}$ ) of the four non-Caryophyllales species  
19 (*Brassica oleracea*, *Helianthus annuus*, *Hordeum vulgare* and *Phlomis lychnitis*) was smaller  
20 than the mean  $[\text{Na}]_{\text{shoot}}$  of the 44 Caryophyllales species studied (Supplementary Data Table  
21 S1) and the rank order of the four non-Caryophyllales species was consistent with the rank  
22 order of  $[\text{Na}]_{\text{shoot}}$  of these species determined by White *et al.* (2017). The  $[\text{Na}]_{\text{shoot}}$  of species  
23 can thus be compared between both studies. In agreement with White *et al.* (2017), the  
24  $[\text{Na}]_{\text{shoot}}$  of all four non-Caryophyllales species was similar to the  $[\text{Na}]_{\text{shoot}}$  of Caryophyllales  
25 species that did not hyperaccumulate  $[\text{Na}]_{\text{shoot}}$ . Root sodium concentrations ( $[\text{Na}]_{\text{root}}$ ) of the

1 four non-Caryophyllales species did not appear to differ from the  $[Na]_{root}$  of Caryophyllales,  
2 indicating that the abnormally large  $[Na]_{shoot}$  among some Caryophyllales did not require the  
3 accumulation of a large  $[Na]_{root}$ .

4

#### 5 *Shoot Na concentrations in Caryophyllales*

6

7 In agreement with White *et al.* (2017),  $[Na]_{shoot}$  of the 44 Caryophyllales species grown in  
8 nonsaline solution could be used to define two distinct  $[Na]_{shoot}$  phenotypes (Fig. 1). The  
9 marginal  $[Na]_{shoot}$  of *Cistanthe grandiflora* and *Psylliostachys suworowi*, in respect to the two  
10  $[Na]_{shoot}$  phenotypes, and their attribution of either phenotype were consistent with White *et*  
11 *al.* (2017). Four *Beta vulgaris* genotypes (sugar beet, beetroot, swiss chard, sea beet), two  
12 *Atriplex* species (*Atriplex halimus*, *A. hortensis*), and three *Amaranthus* species (*Amaranthus*  
13 *caudatus*, *A. cruentus*, *A. tricolor* L.) had similar  $[Na]_{shoot}$ , respectively (Fig. 2,  
14 Supplementary Data Table S1). Thus, there appeared to be little variation in  $[Na]_{shoot}$  among  
15 genotypes of individual Amaranthaceae genera.

16 The threshold between the normal  $[Na]_{shoot}$  and abnormally large  $[Na]_{shoot}$  distributions  
17 differed between the hydroponic experiment presented here and the hydroponic experiments  
18 reported by White *et al.* (2017). Nevertheless, the evolutionary origins of the abnormally  
19 large  $[Na]_{shoot}$  among Caryophyllales coincided (Figs. 2; 3). In addition, log-transformed  
20  $[Na]_{shoot}$  concentrations of the Caryophyllales species represented in the two studies were  
21 strongly correlated (Fig. 4;  $r = 0.97$ ,  $P < 0.001$ ,  $n = 18$  species). The slope of the linear  
22 regression between  $\log_{10}$  transformed  $[Na]_{shoot}$  of Caryophyllales species represented in both  
23 studies was approximately 1, indicating a constant shift in observed  $[Na]_{shoot}$ . Therefore, the  
24 hypothesis (Hypothesis 1 in Introduction) that Caryophyllales species grown hydroponically  
25 in nonsaline solution can be attributed a “normal” or “abnormally large”  $[Na]_{shoot}$  phenotype

1 proposed by White *et al.* (2017) could be confirmed. A nonsaline nutrient solution, in which  
2 the Na reflects only Na contamination of used mineral salts, was used in both studies. A  
3 larger number of plants was grown in the hydroponic experiment presented here by extending  
4 the capacity of an existing hydroponic system by 33% and by using the full capacity of the  
5 system. Thus, the differences in absolute  $[\text{Na}]_{\text{shoot}}$  between studies might reflect changes in  
6 the Na available to plants (Glenn and O’Leary, 1984; Borer *et al.*, 2019). Log-normal  
7  $[\text{Na}]_{\text{shoot}}$  distributions can be used to define abnormally large  $[\text{Na}]_{\text{shoot}}$  among Caryophyllales  
8 species grown in the same environment, but not across environments.

9

#### 10 *Root Na concentrations in Caryophyllales*

11

12 Sodium concentrations in roots of the 44 Caryophyllales species could not be used to define  
13 distinct  $[\text{Na}]_{\text{root}}$  phenotypes and there was little correlation between  $[\text{Na}]_{\text{root}}$  and  $[\text{Na}]_{\text{shoot}}$   
14 among Caryophyllales species (Fig. **1A**). Thus, the evolution of abnormally large  $[\text{Na}]_{\text{shoot}}$   
15 among Caryophyllales did not require the evolution of abnormally large  $[\text{Na}]_{\text{root}}$ . However,  
16  $[\text{Na}]_{\text{shoot}}$  and  $[\text{Na}]_{\text{root}}$  were strongly correlated among hydroponically grown Caryophyllales  
17 species with abnormally large  $[\text{Na}]_{\text{shoot}}$ , but not among species with normal  $[\text{Na}]_{\text{shoot}}$  (Figs. **1;**  
18 **2C**). The hypothesis (Hypothesis 2 in Introduction) that  $[\text{Na}]_{\text{shoot}}$  is correlated with  $[\text{Na}]_{\text{root}}$   
19 among Caryophyllales grown in the same environment could thus be rejected. Sodium is  
20 transported with the transpiration stream via the xylem in plants (Broadley *et al.*, 2012) and  
21 accumulates in transpiring leaves. The wide range of  $[\text{Na}]_{\text{root}}$  compared to the narrow range of  
22  $[\text{Na}]_{\text{shoot}}$  among Caryophyllales species with normal  $[\text{Na}]_{\text{shoot}}$  suggests that some of the  
23 species studied restricted Na uptake into roots, whilst others restricted the translocation of Na  
24 from roots to shoots or actively removed Na from shoots either via the phloem or via salt

1 extrusion mechanisms. Sodium appeared to be partitioned more readily from roots to shoots  
2 among Caryophyllales with abnormal  $[Na]_{shoot}$ .

3

4  *$[Na]_{shoot} / [Na]_{root}$  in Caryophyllales species*

5

6 The distribution of  $[Na]_{shoot} / [Na]_{root}$  quotients from 44 Caryophyllales species could not be  
7 used to define distinct phenotypes (Fig. **1B,D**). However, Caryophyllales species with  
8 abnormally large  $[Na]_{shoot}$  generally had  $[Na]_{shoot} / [Na]_{root}$  above unity, and Caryophyllales  
9 species with normal  $[Na]_{shoot}$  generally had  $[Na]_{shoot} / [Na]_{root}$  below unity (Figs. **1; 2**). A  
10  $[Na]_{shoot} / [Na]_{root}$  quotient above unity suggests that Na is more readily partitioned to the  
11 shoot or that Na removal from the shoot is restricted. The empirical threshold for abnormally  
12 large  $[Na]_{shoot}$  coincided with a  $[Na]_{shoot} / [Na]_{root}$  threshold of unity. A shoot / root  
13 concentration quotient above unity has also been observed for plants that hyperaccumulate  
14 other mineral elements, such as Co, Ni, Mn & Zn (van der Ent *et al.*, 2013). Differences in  
15 environmental conditions, such as heavy metal toxicity or salinity can affect the relative  
16 partitioning of Na between shoots and roots (Patel *et al.*, 1980). Thus,  $[Na]_{shoot} / [Na]_{root}$   
17 quotients might not be useful to define sodium hyperaccumulation in all environments. The  
18 relative partitioning of Na between shoots and roots is, however, likely to be more stable to  
19 environmental perturbation than  $[Na]_{shoot}$ . The hypothesis (Hypothesis 3 in Introduction) that  
20 the relative partitioning of [Na] can be used as an alternative criterion for defining Na  
21 hyperaccumulation in nonsaline environments (cf. van der Ent *et al.*, 2013, for other  
22 elements) could be confirmed.

23

24  *$[Na]_{shoot}$ ,  $[Na]_{root}$  and  $[Na]_{shoot} / [Na]_{root}$  of Caryophyllales families*

25

1 The mean  $[Na]_{shoot}$  of Caryophyllales families when mapped to a phylogeny (Fig. 3), suggests  
2 multiple evolutionary origins of the trait of abnormally large  $[Na]_{shoot}$  in the Caryophyllales  
3 order. In agreement with White *et al.* (2017) and Ievinsh *et al.* (2021), the trait is unlikely to  
4 have evolved in an ancestor of the Amaranthaceae *sensu stricto*, but is common among  
5 species formerly classified Chenopodiaceae. In addition, abnormally large  $[Na]_{shoot}$  is likely  
6 to have evolved in an ancestor of the Aizoaceae and during the evolution of the  
7 Plumbaginaceae. No species in the Montiaceae, Nyctaginaceae, Phytolaccaceae,  
8 Polygonaceae and Portulacaceae accumulated abnormally large  $[Na]_{shoot}$  in the experiment  
9 reported here. White *et al.* (2017) reported that one of the two Portulacaceae species and two  
10 of the 20 Caryophyllaceae species they studied had abnormally large  $[Na]_{shoot}$ . Thus, the trait  
11 of abnormally large  $[Na]_{shoot}$  is not likely to have evolved in ancestors of Montiaceae,  
12 Nyctaginaceae, Phytolaccaceae, Polygonaceae or Portulacaceae, although abnormally large  
13  $[Na]_{shoot}$  may have evolved within the Portulacaceae and Caryophyllaceae.

14 The mean  $[Na]_{shoot}$  of Caryophyllales families were not associated with the mean  $[Na]_{root}$  of  
15 these families (Fig. 3). Thus, the evolution of a large  $[Na]_{shoot}$  among Caryophyllales families  
16 did not require the evolution of a large  $[Na]_{root}$ . However, Caryophyllales families with large  
17  $[Na]_{shoot}$  generally had large  $[Na]_{shoot} / [Na]_{root}$  quotients, suggesting that the accumulation of  
18  $[Na]_{shoot}$  is associated with a distinct partitioning of Na between shoots and roots among  
19 Caryophyllales with large  $[Na]_{shoot}$ .

20

21 *[Na]<sub>shoot</sub>, [Na]<sub>root</sub> and [Na]<sub>shoot</sub> / [Na]<sub>root</sub> of Caryophyllales across environments*

22

23 Data from the literature were sourced to validate the observations based on the hydroponic  
24 experiment described here for different environments (Supplementary Data Table S3). These  
25 data were collected from the papers cited by White *et al.* (2017), which included 29

1 publications that reported data on Na concentrations in either leaves or complete shoots  
2 ( $[\text{Na}]_{\text{top}}$ ) and  $[\text{Na}]_{\text{root}}$  on a DW basis. Experimental details, comprising the type of treatment  
3 (pot, hydroponics, agar, natural) and the Na concentration in the growth medium  
4 ( $[\text{Na}]_{\text{environment}}$ ) were recorded where possible. Different growth conditions (e.g. different  
5 nutrient solutions) within a publication were considered individual studies. In total, 146  
6 unique studies, representing a wide variety of experimental conditions (e.g. nonsaline and  
7 saline conditions, mineral nutrient deficiency, heavy metal toxicity), were sourced from the  
8 literature. The dataset comprised  $[\text{Na}]_{\text{top}}$  and  $[\text{Na}]_{\text{root}}$  data on 39 Caryophyllales species  
9 representing 19 genera and eight families.

10 In agreement with the hydroponic study described here,  $[\text{Na}]_{\text{top}}$  varied more than  $[\text{Na}]_{\text{root}}$   
11 across all Caryophyllales species and environments studied in the literature (Fig. **5A**). The  
12 local regression curves indicated larger  $[\text{Na}]_{\text{top}}$ ,  $[\text{Na}]_{\text{root}}$  and  $[\text{Na}]_{\text{top}} / [\text{Na}]_{\text{root}}$  quotients of Na  
13 hyperaccumulator species ( $n = 109$  measurements) than non-hyperaccumulator species ( $n =$   
14  $69$  measurements). The mean  $[\text{Na}]_{\text{top}}$  of Na hyperaccumulator species ( $94.10 \pm 94.42$  mg Na  
15  $\text{g}^{-1}$  DW) was more than ten times larger than the mean  $[\text{Na}]_{\text{top}}$  of non-hyperaccumulating  
16 species ( $9.29 \pm 14.48$  mg Na  $\text{g}^{-1}$  DW). In comparison, the mean  $[\text{Na}]_{\text{root}}$  of Na  
17 hyperaccumulators ( $22.91 \pm 24.52$  mg Na  $\text{g}^{-1}$  DW) was about four times larger than the mean  
18  $[\text{Na}]_{\text{root}}$  of non-hyperaccumulating species ( $5.31 \pm 8.42$  mg Na  $\text{g}^{-1}$  DW). The mean  $[\text{Na}]_{\text{top}} /$   
19  $[\text{Na}]_{\text{root}}$  quotient of Na hyperaccumulators ( $6.14 \pm 7.73$ ) was three times larger than the mean  
20  $[\text{Na}]_{\text{top}} / [\text{Na}]_{\text{root}}$  quotients of non-hyperaccumulating species ( $2.07 \pm 1.35$ ). Thus, many  
21 Caryophyllales species that do not hyperaccumulate Na in nonsaline environments can have  
22  $[\text{Na}]_{\text{shoot}} / [\text{Na}]_{\text{root}}$  quotients above unity (Fig. **5B**) in some environments. This shows that the  
23  $[\text{Na}]_{\text{shoot}} / [\text{Na}]_{\text{root}}$  threshold of unity did not hold true across all environments. However, the  
24 maximum  $[\text{Na}]_{\text{shoot}} / [\text{Na}]_{\text{root}}$  quotients reached at extreme  $[\text{Na}]_{\text{environment}}$  were much smaller in  
25 non-hyperaccumulator species than in Na hyperaccumulators.

1 The local regression curves of  $[\text{Na}]_{\text{top}}$  against  $[\text{Na}]_{\text{environment}}$  of Na hyperaccumulator species  
2 ( $n = 103$  measurements) and non-hyperaccumulator species ( $n = 46$  measurements) clearly  
3 separated irrespective of the salinity in the environment and despite the variation in  $[\text{Na}]_{\text{top}}$   
4 within both groups (Fig. **6A**). The  $[\text{Na}]_{\text{top}}$  of non-hyperaccumulator species ( $[\text{Na}]_{\text{top}} = \text{ca. } 27$   
5  $\text{mg g}^{-1}$  DW,  $[\text{Na}]_{\text{environment}} = \text{ca. } 200$  mM) plateaued at a much lower  $[\text{Na}]_{\text{environment}}$  than the  
6  $[\text{Na}]_{\text{top}}$  of Na hyperaccumulator species ( $[\text{Na}]_{\text{top}} = \text{ca. } 196$   $\text{mg g}^{-1}$  DW,  $[\text{Na}]_{\text{environment}} = \text{ca. } 666$   
7 mM). Furthermore, the average  $[\text{Na}]_{\text{top}}$  at the salinity threshold previously determined for  
8  $[\text{Na}]_{\text{environment}}$  (20 mM; White *et al.*, 2017) was 7.6  $\text{mg g}^{-1}$  DW in non-hyperaccumulator  
9 species and 40.4  $\text{mg g}^{-1}$  DW for Na hyperaccumulator species, respectively. This further  
10 indicates that the numerical  $[\text{Na}]_{\text{shoot}}$  thresholds derived for individual nonsaline hydroponic  
11 experiments are not universally valid for a range of salinities. Yet, the intercept of non-  
12 hyperaccumulating Caryophyllales at  $[\text{Na}]_{\text{environment}} = 0$  mM ( $[\text{Na}]_{\text{top}} = \text{ca. } 3.2$   $\text{mg g}^{-1}$  DW)  
13 was below the threshold of ca. 4  $\text{mg g}^{-1}$  DW determined by White *et al.* (2017). In  
14 comparison, the intercept for Na hyperaccumulator species ( $[\text{Na}]_{\text{top}} = \text{ca. } 25$   $\text{mg g}^{-1}$  DW) was  
15 much larger than that of non-hyperaccumulator species. The saturation of  $[\text{Na}]_{\text{shoot}}$  among  
16 species grown in saline conditions might explain the low correlation between  $[\text{Na}]_{\text{shoot}}$  and  
17  $[\text{Na}]_{\text{environment}}$  for plants growing in saline, coastal habitats found in the recent study by  
18 Ievinsh *et al.* (2021). Interestingly, the threshold for Na hyperaccumulation in shoots of  
19 species growing on these coastal habitats ( $[\text{Na}]_{\text{shoot}} = 18\text{-}30$   $\text{mg g}^{-1}$  DW,  $[\text{Na}]_{\text{environment}} \geq 200$   
20  $\text{mS m}^{-1}$ ) coincided with the maximum  $[\text{Na}]_{\text{top}}$  of Caryophyllales species that did not  
21 hyperaccumulate Na in their shoots observed here (Fig. **6A**).

22 The local regressions of  $[\text{Na}]_{\text{root}}$  against  $[\text{Na}]_{\text{environment}}$  of Na hyperaccumulator species and  
23 non-hyperaccumulator species overlapped irrespective of  $[\text{Na}]_{\text{environment}}$  (Fig. **6B**). However,  
24 the average  $[\text{Na}]_{\text{root}}$  of hyperaccumulator species was larger than that of non-  
25 hyperaccumulators. This agrees with the observations made in the hydroponic experiment

1 described in this study and suggests that  $[\text{Na}]_{\text{root}}$  alone is not sufficient to define the trait of  
2 Na hyperaccumulation in Caryophyllales.

3 The regression curves of  $[\text{Na}]_{\text{top}} / [\text{Na}]_{\text{root}}$  against  $[\text{Na}]_{\text{environment}}$  of Na hyperaccumulator  
4 species and non-hyperaccumulator species differed, irrespective of  $[\text{Na}]_{\text{environment}}$  (Fig. 6C).  
5 The intercept of the regression of  $[\text{Na}]_{\text{top}} / [\text{Na}]_{\text{root}}$  against  $[\text{Na}]_{\text{environment}}$  ( $[\text{Na}]_{\text{environment}} = 0$   
6 mM) was ca. 1.2 / 1 for non-hyperaccumulator species, and the 95 % confidence interval (CI)  
7 at the intercept included unity (Fig. 6D). In comparison, the intercept of the regression of  
8  $[\text{Na}]_{\text{top}} / [\text{Na}]_{\text{root}}$  against  $[\text{Na}]_{\text{environment}}$  for Na hyperaccumulator species was ca. 6.4 / 1 and the  
9 95 % CI did not include unity. This is consistent with the observations made in our  
10 hydroponic experiment. The average  $[\text{Na}]_{\text{top}} / [\text{Na}]_{\text{root}}$  quotients of Na hyperaccumulators and  
11 non-hyperaccumulators at  $[\text{Na}]_{\text{environment}} = 20$  mM were ca. 6.9 / 1 and 1.9 / 1, respectively.  
12 This suggests, that  $[\text{Na}]_{\text{top}} / [\text{Na}]_{\text{root}}$  quotients in Caryophyllales may increase with increasing  
13  $[\text{Na}]_{\text{environment}}$ . The  $[\text{Na}]_{\text{top}} / [\text{Na}]_{\text{root}}$  quotients of Na hyperaccumulator species and non-  
14 hyperaccumulator species plateaued at ca. 7.2 / 1 and 2.5 / 1, respectively. Thus, a  $[\text{Na}]_{\text{top}} /$   
15  $[\text{Na}]_{\text{root}}$  threshold above unity might be more conservative for defining Na hyperaccumulation  
16 among Caryophyllales species across environments.

17 It is worth highlighting that the reported values of  $[\text{Na}]_{\text{environment}}$  in individual studies may not  
18 be precise. Thus, even  $[\text{Na}]_{\text{environment}}$  of 0 mM may not be exactly zero due to Na  
19 contamination, as shown by our own hydroponic experiments. Yet, the observations from  
20 hydroponic experiments using nonsaline nutrient solution described here and previously  
21 (White *et al.*, 2017) are generally in agreement with the literature. Thus, the hypothesis  
22 (Hypothesis 4 in Introduction) that observations made for Caryophyllales grown  
23 hydroponically under nonsaline conditions can be generalised across environments was not  
24 rejected. However, the exact numerical thresholds inferred in individual studies may differ  
25 because the uptake and partitioning of Na by Caryophyllales can change as a consequence of

1 different mineral nutrition and with different salinity, in particular. Mineral nutrient  
2 deficiencies and toxicities of mineral elements are common in both natural and agricultural  
3 environments (White *et al.*, 2013). Evidence was provided here, that differences in  $[\text{Na}]_{\text{shoot}}$   
4 and  $[\text{Na}]_{\text{shoot}} / [\text{Na}]_{\text{root}}$  between Caryophyllales species that hyperaccumulate Na and those  
5 that do not can be observed across environments (Figs. 4; 5; 6). Using  $[\text{Na}]_{\text{shoot}} / [\text{Na}]_{\text{root}}$   
6 quotients in addition to  $[\text{Na}]_{\text{shoot}}$  to identify Na hyperaccumulator species in the  
7 Caryophyllales may be preferable when studying Caryophyllales grown in their natural  
8 environments, without controlled mineral nutrition. Provided clean roots can be obtained  
9 from the substrate, this may enable the study of the evolution of Na hyperaccumulation  
10 among Caryophyllales that are difficult or slow to grow in controlled environments, such as  
11 Cactaceae. Abnormally large  $[\text{Na}]_{\text{shoot}}$  has previously been reported for some Cactaceae  
12 grown in nonsaline environments (White *et al.*, 2017). No Cactaceae species were grown  
13 hydroponically in the experiment reported here due to their slow growth rates. Future studies  
14 could test the prevalence of Na hyperaccumulation among Cactaceae and other  
15 Caryophyllales that are difficult to grow hydroponically by characterising their  $[\text{Na}]_{\text{shoot}}$  as  
16 well as their  $[\text{Na}]_{\text{shoot}} / [\text{Na}]_{\text{root}}$  quotients.

17 The genetic mechanisms controlling the hyperaccumulation of Na in nonsaline conditions  
18 remain unknown. Sodium homeostasis in plants is likely to be controlled by many genes  
19 (Zhang *et al.*, 2017). Nevertheless, experiments in the model plant *Arabidopsis thaliana* (L.)  
20 Heynh. indicate that *AtHKT1* and *AtSOS1* like genes could be candidate genes contributing to  
21 the trait of Na hyperaccumulation. *HKT1* is expressed in both roots and leaves in *arabidopsis*  
22 and *athkt1* knockout mutants have smaller  $[\text{Na}]_{\text{root}}$  but larger  $[\text{Na}]_{\text{shoot}}$  and thus larger  $[\text{Na}]_{\text{shoot}}$   
23 /  $[\text{Na}]_{\text{root}}$  quotients (Mäser *et al.*, 2002). Conversely, greater expression of *AtHKT1;1* in roots  
24 may cause smaller  $[\text{Na}]_{\text{shoot}}$  due to its proposed role in retrieving Na from the xylem sap (Jha  
25 *et al.*, 2010). *HKT1* type genes are also involved in Na homeostasis in other angiosperms,

1 including tomato (Jaime-Pérez *et al.*, 2017), wheat (James *et al.*, 2011) and the halophytic  
2 grass *Puccinellia tenuiflora* (Griseb.) Scribn. & Merr. (Zhang *et al.*, 2017). The ability to  
3 hyperaccumulate Na in shoots would imply greater tolerance for Na in shoot tissues, although  
4 this has not been demonstrated formally here.

5

#### 6 *Associations between Na hyperaccumulation and the accumulation of other elements*

7

8 The accumulation of Na<sup>+</sup> in shoots requires the accumulation of negatively charged  
9 counterions, such as Cl<sup>-</sup>, sulfate, nitrate, phosphate, or organic anions. However, the distinct  
10 phenotype of abnormally large [Na]<sub>shoot</sub> in Caryophyllales species studied here and by White  
11 *et al.* (2017) did not appear to be associated with abnormally large shoot concentrations of Cl,  
12 S, N or P (Neugebauer *et al.*, 2018; Table 2). Similarly, the accumulation of abnormally large  
13 [Na]<sub>shoot</sub> did not appear to be associated with abnormally large concentrations of K, Ca or Mg  
14 in shoots (Neugebauer *et al.*, 2018; Table 2). Species in the Caryophyllales often have greater  
15 shoot / root concentrations of P, K, Mg, Fe, Mn, Cu, Zn and Ni than other angiosperms  
16 (Neugebauer, 2019). However, although these elements showed variation in their shoot / root  
17 concentrations among Caryophyllales species this was not associated with large  
18 concentrations of these elements in the shoot (Neugebauer, 2019).

19

#### 20 *Conclusions*

21

22 Two distinct Caryophyllales [Na]<sub>shoot</sub> phenotypes, “normal [Na]<sub>shoot</sub>” and “abnormally large  
23 [Na]<sub>shoot</sub>”, can be defined. Species that exhibited normal [Na]<sub>shoot</sub> or abnormally large [Na]<sub>shoot</sub>  
24 in the experiment presented here also exhibited normal [Na]<sub>shoot</sub>, or abnormally large [Na]<sub>shoot</sub>  
25 in the experiment presented by White *et al.* (2017). However, the [Na]<sub>shoot</sub> threshold defining

1 Na hyperaccumulation differed between the two studies. This demonstrates that a numerical  
2 threshold for defining abnormally large  $[\text{Na}]_{\text{shoot}}$  only applies to species grown under the  
3 same conditions. The  $[\text{Na}]_{\text{shoot}}$  of Caryophyllales species was not correlated with their  $[\text{Na}]_{\text{root}}$   
4 and the evolution of abnormally large  $[\text{Na}]_{\text{shoot}}$  did not appear to require the evolution of a  
5 large  $[\text{Na}]_{\text{root}}$ . However, species with abnormally large  $[\text{Na}]_{\text{shoot}}$  generally have  $[\text{Na}]_{\text{shoot}} /$   
6  $[\text{Na}]_{\text{root}}$  quotients greater than unity when grown under nonsaline conditions and  $[\text{Na}]_{\text{shoot}} /$   
7  $[\text{Na}]_{\text{root}}$  quotients can thus be used as an additional measure to define species that  
8 hyperaccumulate Na. The prevalence of Na hyperaccumulation in Caryophyllales families  
9 that are difficult to grow under controlled conditions, such as Cactaceae, remains unknown  
10 but may be explored using  $[\text{Na}]_{\text{shoot}} / [\text{Na}]_{\text{root}}$  to confirm Na hyperaccumulation.

11

## 12 SUPPLEMENTARY DATA

13

14 Supplementary data are available online at <https://academic.oup.com/aob> and consist of the  
15 following. **Table S1** Taxonomy of the 52 angiosperm genotypes grown hydroponically in  
16 nonsaline solution, their allocated shoot sodium (Na) phenotype, number of days grown  
17 hydroponically, shoot and root fresh and dry weights, and their shoot and root Na  
18 concentrations. The commercial supplier, synonymous names given by the supplier (Supplier  
19 Synonym for Species), and additional names or descriptions including variety and cultivar  
20 (Additional Name) are provided. **Table S2** Complete and partial matK sequences used for  
21 inferring the phylogeny of the 13 Caryophyllales families represented by 44 Caryophyllales  
22 species and matK sequences of the four non-Caryophyllales species used for rooting the  
23 phylogenetic tree. **Table S3** Sodium (Na) concentrations in tissues of Caryophyllales species  
24 sourced from the literature. The taxonomic affiliations, allocation of Na hyperaccumulator  
25 phenotype and Reference IDs are based on White *et al.* (2017). Sodium concentrations in

1 plants are expressed on a dry weight basis for individual organs (shoot, leaf, root) and sodium  
2 concentrations in the growth medium (Na\_env\_mM) are expressed as mM. **Table S4**  
3 Analysis of variance (ANOVA) table for sodium concentrations in shoots and roots (Organ)  
4 of three genotypes (*Beta vulgaris* L., *Helianthus annuus* L., *Hordeum vulgare* L.) grown in  
5 two groups of four gullies, each supplied by nutrient solution from a different tank (Tank)  
6 and both divided into two blocks (Block). Presented are the sums of squares (Sum Sq),  
7 means squares (Mean Sq), F-values and probability values. \*\*\* indicates  $P < 0.001$  and \*\*  
8 indicates  $P < 0.01$ , respectively.

9

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16

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18

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21 interpreted the data. K.N. and P.J.W. drafted the manuscript. All authors commented on the  
22 manuscript.

23

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- 11

1 Figure Captions

2

3 Fig. 1: Mean sodium concentrations (A) in shoots ( $[Na]_{shoot}$ ) and in roots ( $[Na]_{root}$ ) of 44  
4 Caryophyllales species grown in nonsaline solution, (B) mean  $[Na]_{shoot}$  of the same species  
5 plotted against their respective  $[Na]_{shoot} / [Na]_{root}$  quotients, (C)  $\log_{10}$  transformed  $[Na]_{shoot}$   
6 and  $[Na]_{root}$ , and (D)  $\log_{10}$  transformed  $[Na]_{shoot}$  plotted against  $[Na]_{shoot} / [Na]_{root}$  quotients.  
7 Vertical dashed red lines indicate  $[Na]_{shoot} = 10^{-0.1}$  or  $[Na]_{shoot} \log_{10} = -0.1$ . Diagonal dashed  
8 lines and horizontal dashed lines indicate  $[Na]_{shoot} / [Na]_{root}$  of unity. Red solid lines at the  
9 plot margins indicate empirical distribution functions based on kernel density estimates. Data  
10 for individual species can be found in Supplementary Data Table S1.

11

12 Fig. 2: Mean sodium concentrations in shoots ( $[Na]_{shoot}$ ) and in roots ( $[Na]_{root}$ ) of (A) 47  
13 Caryophyllales genotypes grown in nonsaline solution. Closed symbols and “+” indicate  
14 genotypes with abnormally large  $[Na]_{shoot}$  and open symbols indicate genotypes with normal  
15  $[Na]_{shoot}$ . Mean  $[Na]_{shoot} / [Na]_{root}$  quotients of (B) the 13 Caryophyllales genotypes with  
16 abnormally large  $[Na]_{shoot}$  and (C) the 34 Caryophyllales genotypes with normal  $[Na]_{shoot}$ ,  
17 respectively. Colours indicate Aizoaceae (black), Amaranthaceae (red), Basellaceae (orange),  
18 Caryophyllaceae (light blue), Montiaceae (green), Nyctaginaceae (yellow), Petiveriaceae  
19 (dark blue), Phytolaccaceae (dark grey), Plumbaginaceae (dark pink), Polygonaceae (brown),  
20 Portulacaceae (cyan), Simmondsiaceae (light pink) and Talinaceae (light grey). Multiple  
21 genotypes of an Amaranthaceae species are indicated by circles, squares and “+”,  
22 respectively. The red dashed line indicates a  $[Na]_{shoot} / [Na]_{root}$  quotient of unity. The black  
23 lines indicate the correlation between  $[Na]_{shoot}$  and  $[Na]_{root}$  among Caryophyllales with  
24 abnormally large  $[Na]_{shoot}$  (solid,  $r = 0.82$ ,  $P < 0.001$ ,  $n = 13$  genotypes) and normal  $[Na]_{shoot}$

1 (dashed,  $r = -0.001$ ,  $P = 0.996$ ,  $n = 34$ ), respectively. Data for individual species can be found  
2 in Supplementary Data Table S1.

3

4 Fig. 3: Phylogenetic relationships among 13 Caryophyllales families based on matK. Mean  
5  $\log_{10}$  transformed shoot sodium ( $[\text{Na}]_{\text{shoot}}$ ) concentrations and  $[\text{Na}]_{\text{shoot}} / \text{root Na}$   
6 concentration ( $[\text{Na}]_{\text{root}}$ ) quotients (A), and mean  $[\text{Na}]_{\text{root}}$  (B) are mapped to the phylogeny  
7 using maximum likelihood interpolation. The relative lengths of the scale bars are the same  
8 for each phylogeny. Numbers at the tips indicate untransformed family mean values and their  
9 colours indicate the  $\log_{10}$  transformed family mean values as shown in the colour scale bars.

10

11 Fig. 4: Shoot sodium concentrations ( $[\text{Na}]_{\text{shoot}}$ ) of Caryophyllales species represented in the  
12 present study and that of White *et al.* (2017). The linear regression (black) between both  
13 datasets is  $y = -0.582 + 1.039 x$ , with  $y$  and  $x$  indicating the  $\log_{10}([\text{Na}]_{\text{shoot}})$  of the data  
14 presented by White *et al.* (2017) and the present study, respectively. The red line indicates  $y$   
15  $= 0 + 1 x$ .

16

17 Fig. 5: Concentrations of sodium in whole shoots or leaves ( $[\text{Na}]_{\text{top}}$ ) and in roots ( $[\text{Na}]_{\text{root}}$ )  
18 expressed on a dry weight (DW) basis sourced from the literature (Supplementary Data Table  
19 S3). The dataset contained 39 Caryophyllales species from eight families and 146 studies (A).  
20 Two measurements of *Suaeda monoica* (Amaranthaceae) with  $[\text{Na}]_{\text{root}} > 100 \text{ mg g}^{-1} \text{ DW}$   
21 ( $[\text{Na}]_{\text{top}} = 168.8 \text{ mg g}^{-1} \text{ DW}$ ,  $198.1 \text{ mg g}^{-1} \text{ DW}$ ;  $[\text{Na}]_{\text{root}} = 116.0 \text{ mg g}^{-1} \text{ DW}$ ,  $178.3 \text{ mg g}^{-1}$   
22  $\text{DW}$ ) are not shown. Colours indicate the unambiguous Na hyperaccumulators (red) and non-  
23 hyperaccumulators (blue) proposed by White *et al.* (2017). The fitted curves and 95 %  
24 confidence intervals are based on locally estimated scatterplot smoothing (LOESS) and  
25 include the measurements that are not shown. Symbols indicate whole shoots (triangles) and

1 leaves (circles). The solid black line indicates a  $[\text{Na}]_{\text{top}} / [\text{Na}]_{\text{root}}$  quotient of unity and the  
2 dashed black line indicates  $[\text{Na}]_{\text{top}} = 4 \text{ mg g}^{-1} \text{ DW}$ . A subset of the same data and regression  
3 is presented in panel (B), showing the approximate point of separation (green lines) between  
4 Na hyperaccumulators and non-hyperaccumulators at  $[\text{Na}]_{\text{shoot}} = 44.0 \text{ mg g}^{-1} \text{ DW}$  and  $[\text{Na}]_{\text{root}}$   
5  $= 16.8 \text{ mg g}^{-1} \text{ DW}$ .

6

7 Fig. 6: Concentrations of sodium in (A) complete shoots or leaves ( $[\text{Na}]_{\text{top}}$ ) and in (B) roots  
8 ( $[\text{Na}]_{\text{root}}$ ) on a dry weight (DW) basis, and  $[\text{Na}]_{\text{top}} / [\text{Na}]_{\text{root}}$  quotients (C, D) plotted against  
9 the Na concentration in the environment ( $[\text{Na}]_{\text{environment}}$ ), respectively. Data are sourced from  
10 the literature (Supplementary Data Table S3) and represent 33 Caryophyllales species from  
11 seven families and 127 studies. One measurement of *Suaeda monoica* (Amaranthaceae) with  
12  $[\text{Na}]_{\text{root}} > 150 \text{ mg g}^{-1} \text{ DW}$  ( $[\text{Na}]_{\text{top}} = 198.1 \text{ mg g}^{-1} \text{ DW}$ ;  $[\text{Na}]_{\text{root}} = 178.3 \text{ mg g}^{-1} \text{ DW}$ ) is not  
13 shown in (B) and one measurement of *Atriplex hymenelytra* (Amaranthaceae) with  $[\text{Na}]_{\text{top}} /$   
14  $[\text{Na}]_{\text{root}} = 53.7$  is not shown in (C). Colours indicate unambiguous hyperaccumulators (red)  
15 and non-hyperaccumulators (blue) identified by White *et al.* (2017). The fitted curves and 95  
16 % confidence intervals are based on locally estimated scatterplot smoothing (LOESS) and  
17 include the measurements that are not shown. Symbols indicate complete shoots (triangles)  
18 and leaves (circles). The vertical dashed lines indicate  $[\text{Na}]_{\text{environment}} = 20 \text{ mM}$  and the  
19 horizontal dashed lines indicate  $[\text{Na}]_{\text{top}} = 4 \text{ mg g}^{-1} \text{ DW}$  and  $[\text{Na}]_{\text{top}} / [\text{Na}]_{\text{root}}$  of unity,  
20 respectively.

21

1 Table 1: Species grown hydroponically and their taxonomic affiliations. Species marked with  
 2 a “\*” were grown hydroponically in both this study and in the experiments reported by White  
 3 *et al.* (2017).

<b>Order</b>	<b>Family</b>	<b>Number of Genera</b>	<b>Number of Species</b>	<b>Species</b>
Asterales	Asteraceae	1	1	<i>Helianthus annuus</i> *
Brassicales	Brassicaceae	1	1	<i>Brassica oleracea</i> *
Poales	Poaceae	1	1	<i>Hordeum vulgare</i> *
Lamiales	Lamiaceae	1	1	<i>Phlomis lychnitis</i>
Caryophyllales	Aizoaceae	7	7	<i>Bergeranthus vespertinus</i> , <i>Carpobrotus edulis</i> *, <i>Delosperma cooperi</i> *, <i>Dorotheanthus bellidiformis</i> *, <i>Lampranthus</i> spp., <i>Pleiospilos nelii</i> , <i>Tetragonia tetragonioides</i>
Caryophyllales	Amaranthaceae	4	7	<i>Amaranthus caudatus</i> *, <i>Amaranthus cruentus</i> *, <i>Amaranthus tricolor</i> , <i>Atriplex halimus</i> , <i>Atriplex hortensis</i> *, <i>Beta vulgaris</i> *, <i>Salicornia europaea</i>
Caryophyllales	Basellaceae	1	1	<i>Basella alba</i>
Caryophyllales	Caryophyllaceae	10	10	<i>Agrostemma githago</i> *, <i>Cerastium tomentosum</i> *

				<i>Dianthus glacialis</i> , <i>Gypsophila pacifica</i> , <i>Herniaria glabra</i> , <i>Melandrium keiskei</i> , <i>Petrorhagia prolifera</i> , <i>Sagina subulata</i> *, <i>Silene armeria</i> *, <i>Stellaria media</i>
Caryophyllales	Montiaceae	3	3	<i>Claytonia perfoliata</i> , <i>Montiopsis umbellata</i> , <i>PheMERanthus teretifolius</i>
Caryophyllales	Nyctaginaceae	1	1	<i>Mirabilis longiflora</i>
Caryophyllales	Petiveriaceae	1	1	<i>Petiveria alliacea</i>
Caryophyllales	Phytolaccaceae	1	1	<i>Phytolacca americana</i> *
Caryophyllales	Plumbaginaceae	4	4	<i>Armeria maritima</i> *, <i>Limonium sinuatum</i> *, <i>Plumbago auriculata</i> *, <i>Psylliostachys suworowi</i> *
Caryophyllales	Polygonaceae	7	7	<i>Antigonon leptopus</i> , <i>Emex australis</i> , <i>Eriogonum arborescens</i> , <i>Fagopyrum esculentum</i> , <i>Persicaria capitata</i> *, <i>Rheum palmatum</i> *, <i>Rumex sanguineus</i>
Caryophyllales	Portulacaceae	1	1	<i>Cistanthe grandiflora</i>
Caryophyllales	Simmondsiaceae	1	1	<i>Simmondsia chinensis</i>
Caryophyllales	Talinaceae	1	1	<i>Talinum paniculatum</i>

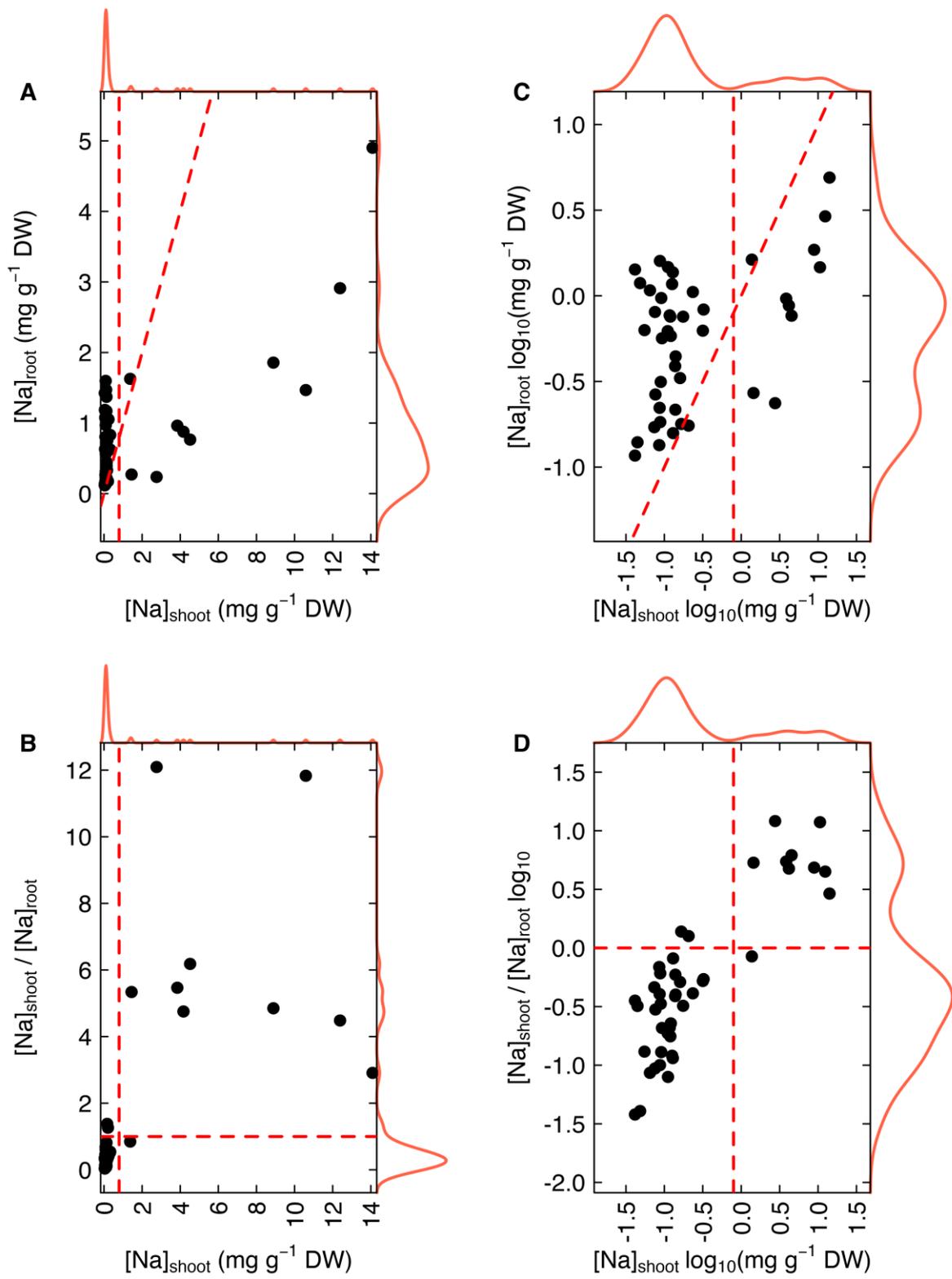
1 Table 2: Mean log<sub>e</sub>-transformed element concentrations (mg g<sup>-1</sup> dry weight) and 95%  
2 confidence intervals (95% CI) in shoots of Caryophyllales species reported by Neugebauer *et*  
3 *al.* (2018). Species, for which the elements sodium (Na), chlorine (Cl), sulfur (S), nitrogen  
4 (N), phosphorous (P), potassium (K), magnesium (Mg) and calcium (Ca) were measured,  
5 were grouped into those with abnormally large Na concentrations (hyper) and those with  
6 normal Na concentrations (normal), respectively, as identified by White *et al.* (2017).

7

<b>Element</b>	<b>Type</b>	<b>Mean [95% CI]</b>	<b>Species</b>
Na	normal	-0.87 [-1.08, -0.66]	49
	hyper	2.78 [2.36, 3.21]	12
Cl	normal	1.48 [1.35, 1.61]	22
	hyper	1.37 [1.14, 1.60]	7
S	normal	1.29 [1.18, 1.39]	22
	hyper	1.44 [1.25, 1.62]	7
N	normal	3.96 [3.89, 4.03]	38
	hyper	3.87 [3.73, 4.01]	9
P	normal	2.14 [2.01, 2.28]	49
	hyper	2.22 [1.94, 2.49]	12
K	normal	3.68 [3.57, 3.79]	49
	hyper	3.90 [3.68, 4.12]	12
Mg	normal	1.72 [1.58, 1.85]	49
	hyper	1.96 [1.69, 2.23]	12
Ca	normal	2.25 [2.11, 2.39]	49
	hyper	2.19 [1.89, 2.48]	12

8

1 Fig. 1

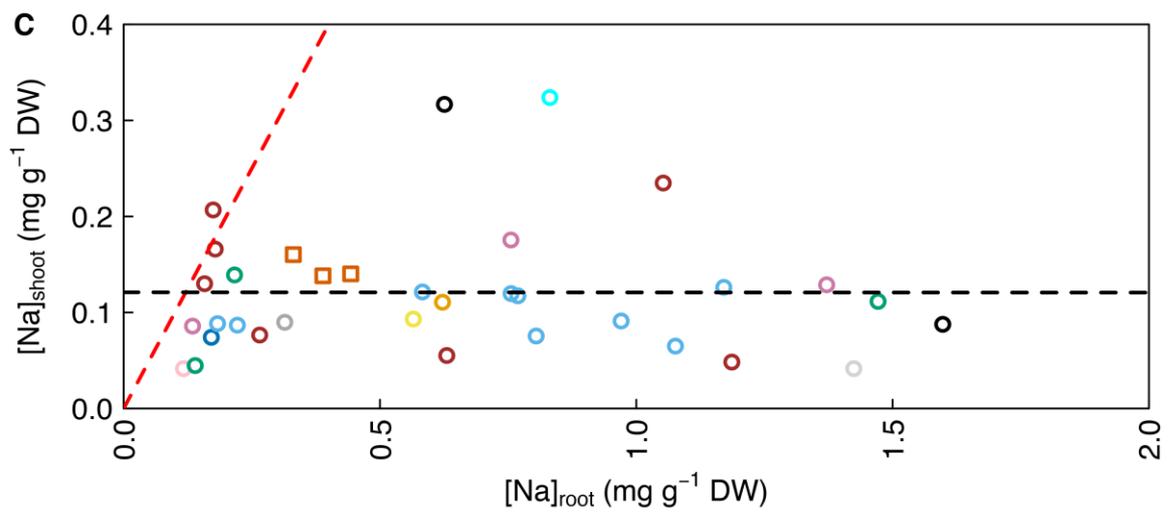
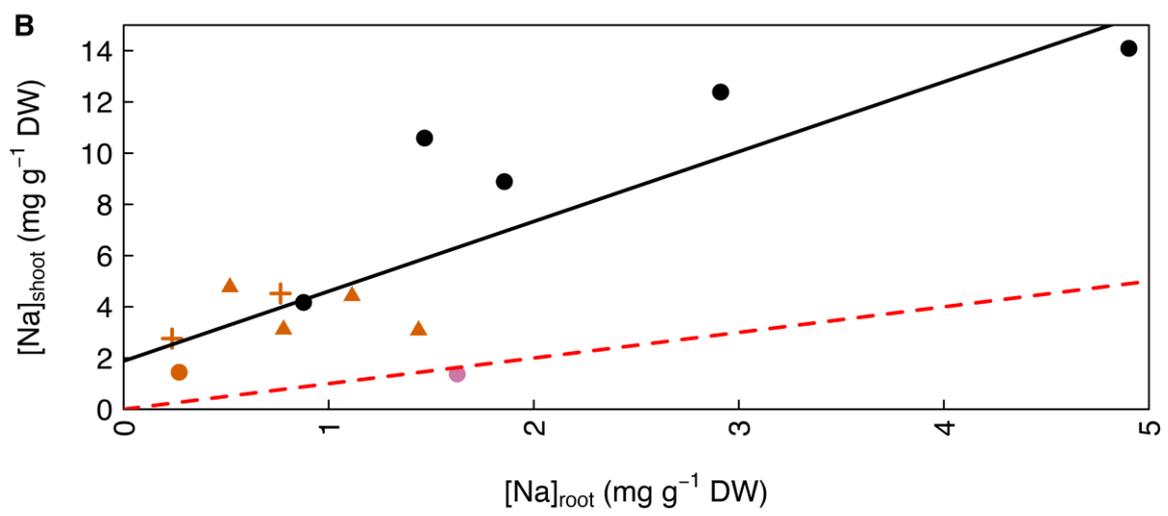
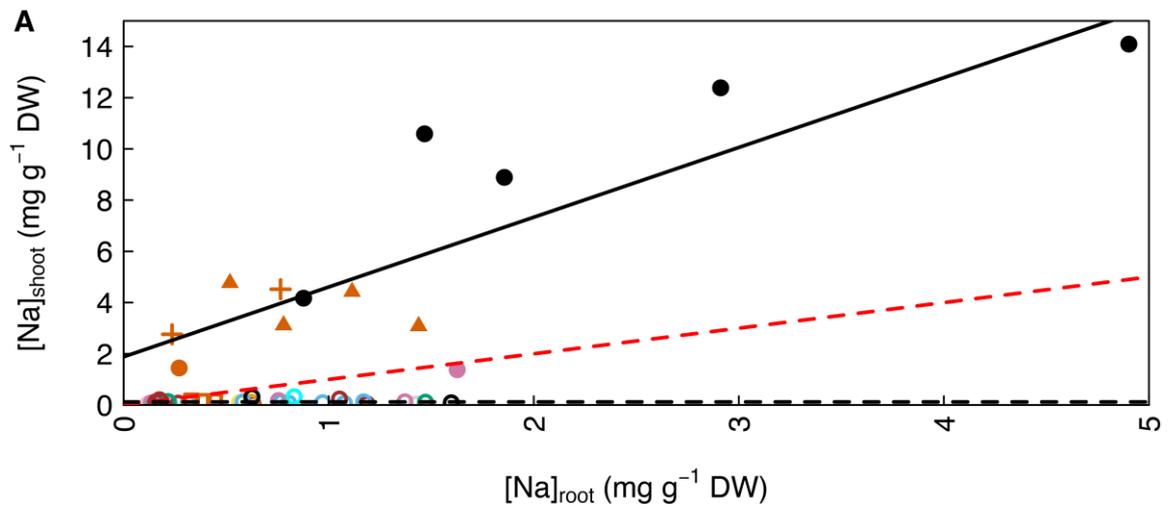


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1 Fig. 2

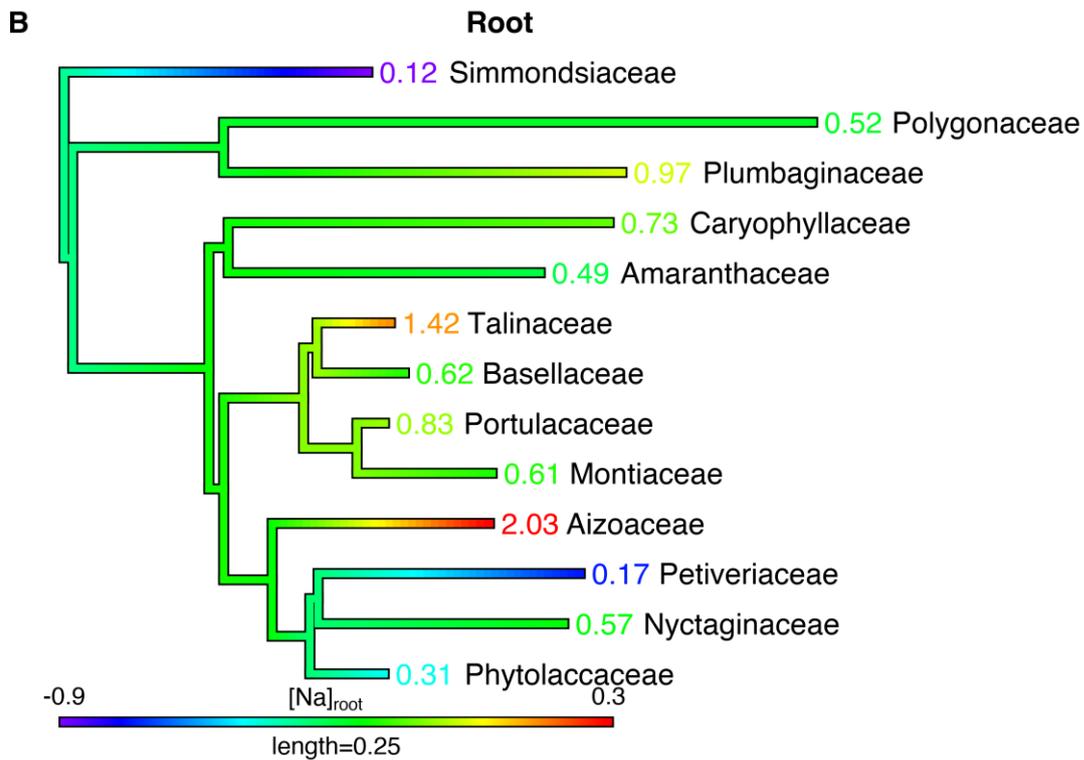
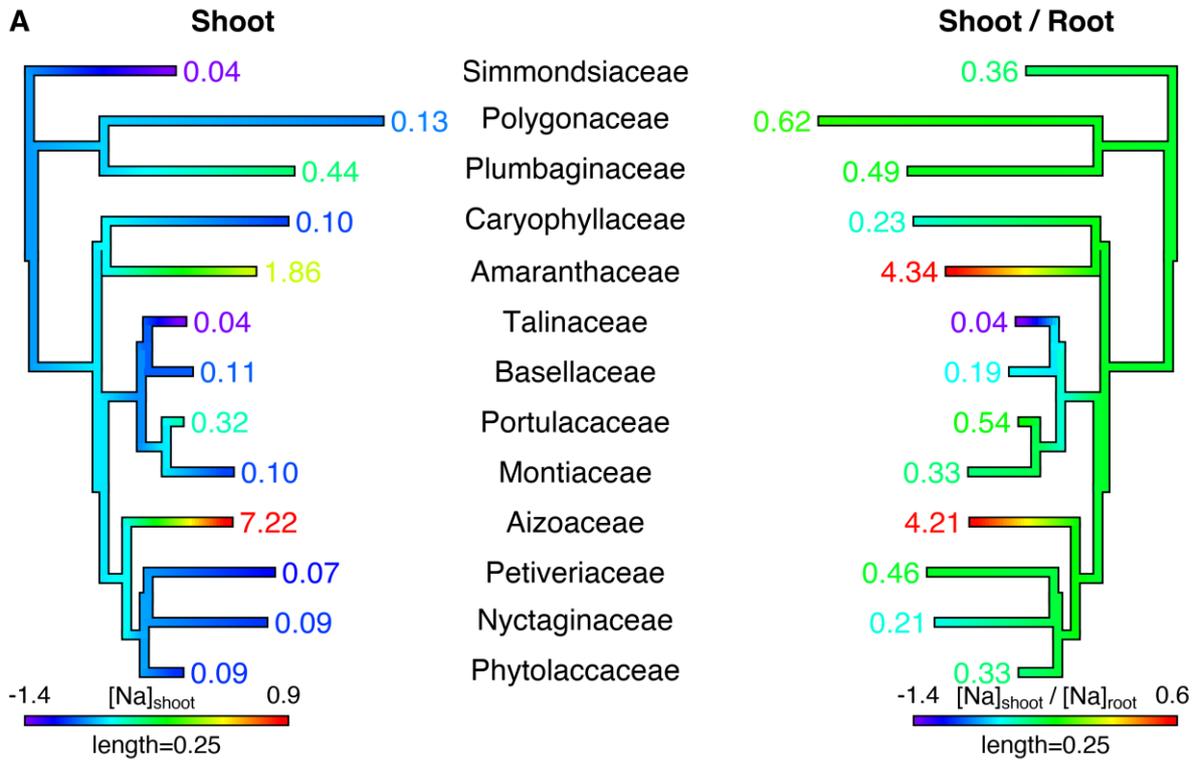
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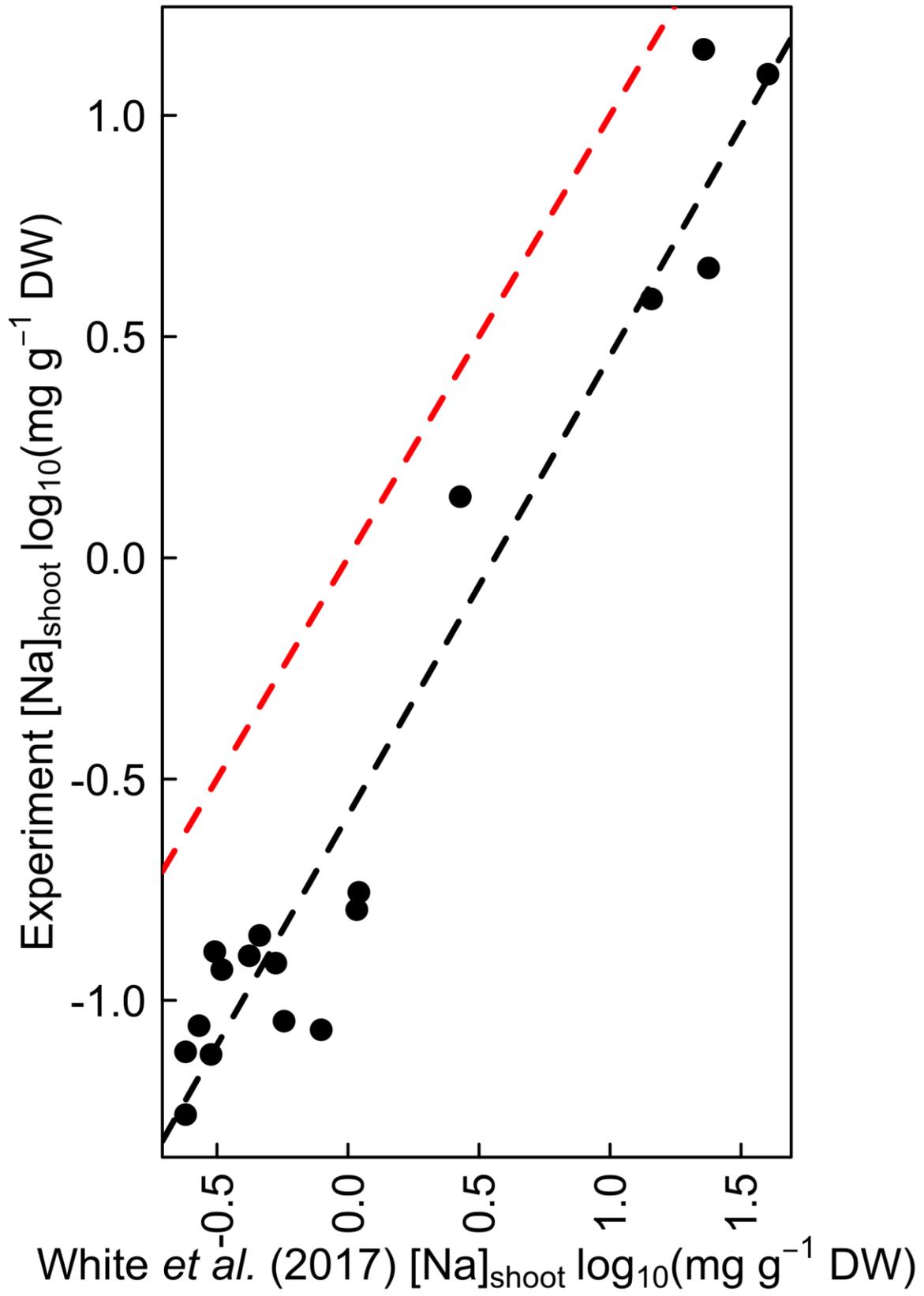
1 Fig 3



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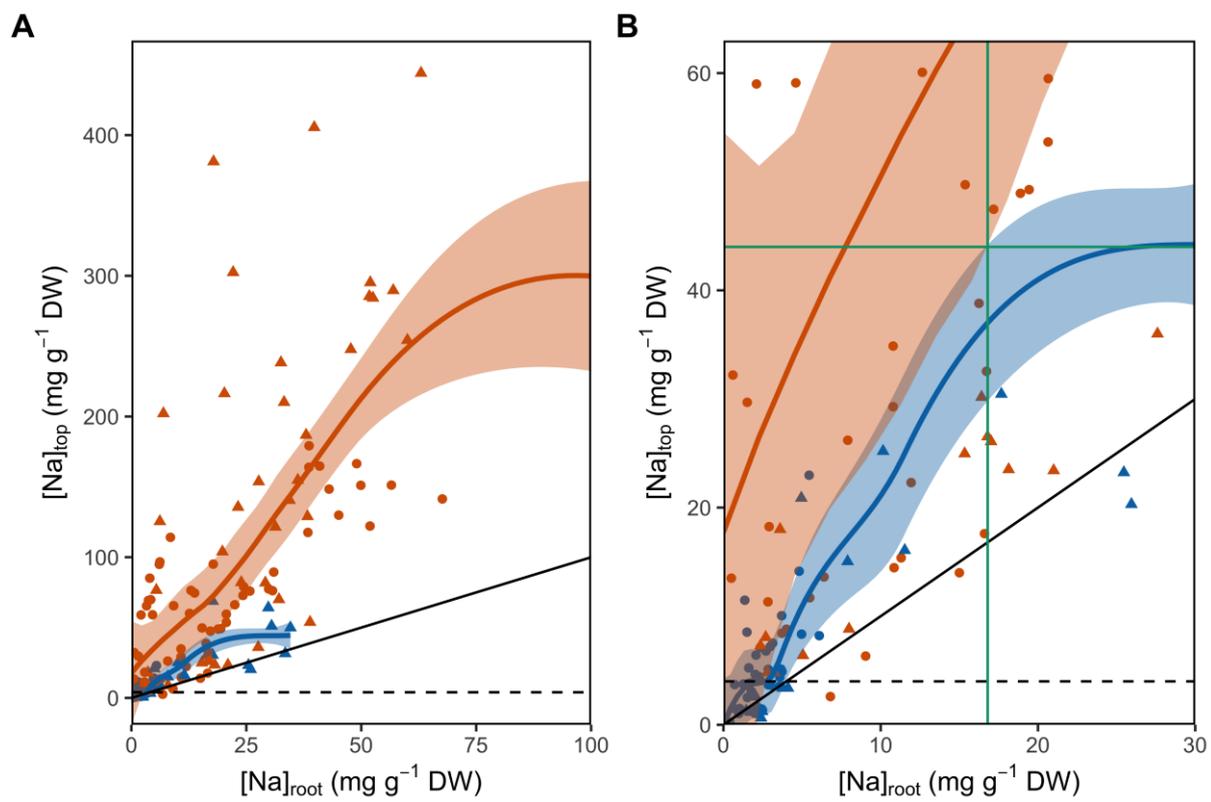
1 Fig 4



2

3

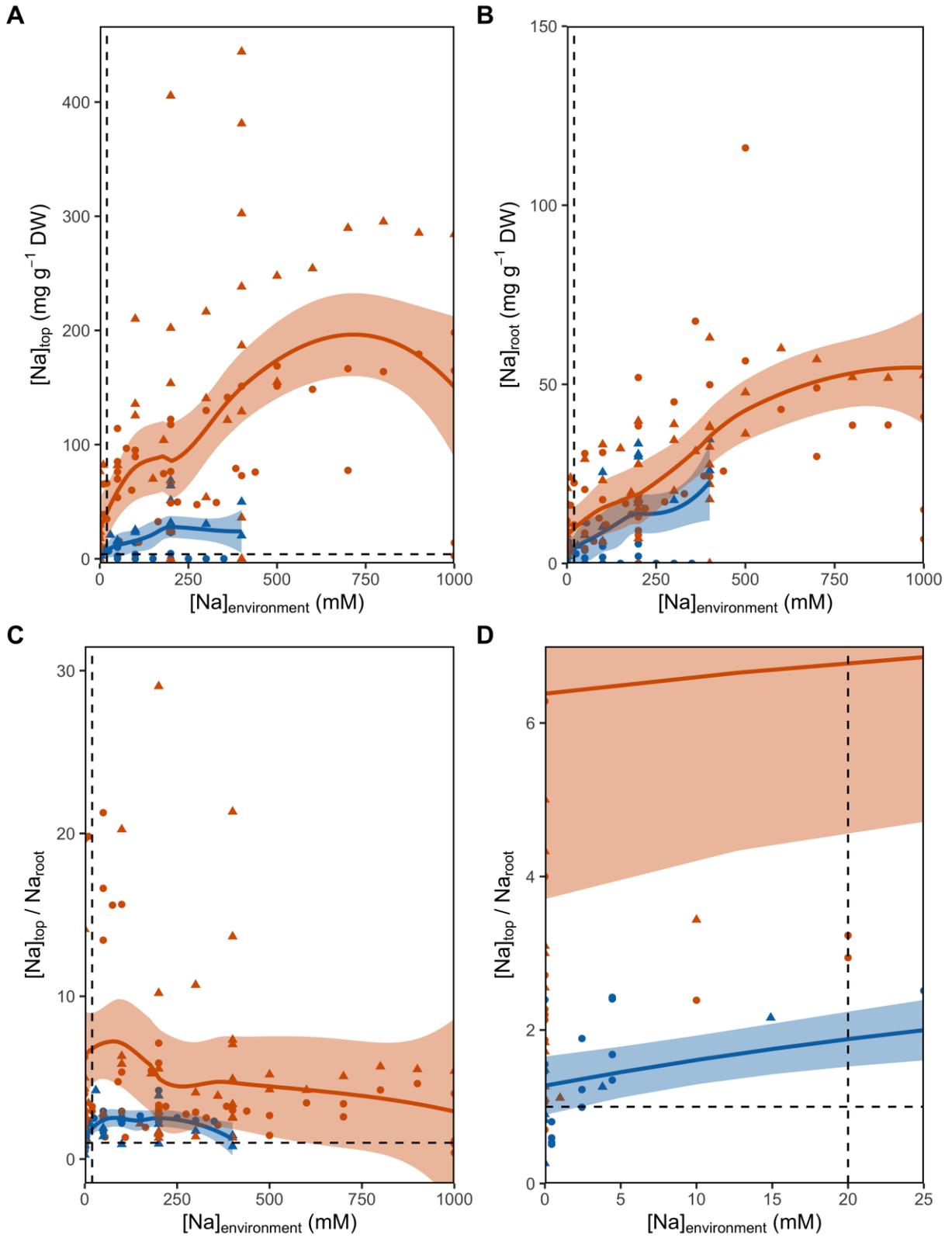
1 Fig 5



2

3

1 Fig 6



2