2

3 Original Article

4

5 Title:

6 Predicting species' tolerance to salinity and alkalinity using distribution data and

7 geochemical modelling: a case study using Australian grasses

8

9 C. Haris Saslis-Lagoudakis[1,*,†], Xia Hua[1], Elisabeth Bui[2], Camile Moray[1], and Lindell

10 Bromham[1]

11

12 1 Centre for Macroevolution and Macroecology, Research School of Biology, Australian

13 National University, Canberra, Australian Capital Territory 0200, Australia

14 2 CSIRO Land and Water, GPO Box 1666, Canberra, Australian Capital Territory 2601,

15 Australia

16

17 Running title:

18 Using geochemical modelling to predict salt and alkalinity-tolerance

19

20 *Corresponding author: C.H.SaslisLagoudakis@gmail.com

21

22 †Current address: Evolutionary Genomics Section, Natural History Museum of Denmark,

23 University of Copenhagen, 1307 Copenhagen, Denmark

1   **Abstract**

2   • Background and Aims

3   Salt tolerance has evolved many times independently in different plant groups. One possible

4   explanation for this pattern is that it builds upon a general suite of stress-tolerance traits. If

5   this is the case, then we might expect a correlation between salt tolerance and other tolerances

6   to different environmental stresses. This association has been hypothesised for salt and

7   alkalinity-tolerance. However, a major limitation in investigating large-scale patterns of these

8   tolerances is that lists of known tolerant species are incomplete. Here, we explore whether we

9   can predict species' salt and alkalinity-tolerance using geochemical modelling for Australian

10   grasses. Then, we assess the correlation between taxa found in conditions of high predicted

11   salinity and alkalinity.

12   • Methods

13   We use extensive occurrence data for Australian grasses and geochemical modelling to

14   predict values of pH and electrical conductivity (EC) to which species are exposed in their

15   natural distributions. Using parametric and phylogeny-corrected tests, we i) evaluate our

16   geochemical predictions using a list of known halophytes as a control, and ii) ask whether

17   taxa that occur in conditions of high predicted salinity are also found in conditions of high

18   predicted alkalinity.

19   • Key Results

20   We show that genera containing known halophytes have higher predicted salinity conditions

21   than those not containing known halophytes. Additionally, we find that taxa occurring in high

22   predicted salinity tend to also occur in high predicted alkalinity.

23   • Conclusions

24   Geochemical modelling using species' occurrence data is a potentially useful approach to

25   predict species' relative natural tolerance to challenging environmental conditions. Our

findings also demonstrate a correlation between salinity and alkalinity-tolerance. Further investigations can consider the phylogenetic distribution of specific traits involved in these ecophysiological strategies, ideally by incorporating more complete finer scale geochemical information, as well as laboratory experiments.

Keywords: alkalinity-tolerance, geochemical modelling, macroevolution, phylogeny, Poaceae, salt tolerance, stress resistance syndrome

1 **Introduction**

2   Many plant species have developed several ecophysiological strategies to tolerate

3 extreme conditions in challenging environments. For example, species that complete their life

4 cycle in saline environments - known as halophytes - have evolved various mechanisms that

5 have enabled them to survive and reproduce in these environments (Flowers & Colmer, 2008;

6 Munns & Tester, 2008). These mechanisms are related to water uptake and defence against

7 ion toxicity within the plant, such as the accumulation and compartmentalisation of saline

8 ions, the ability to limit the entry of these ions into the transpiration stream, the synthesis of

9 compatible solutes for osmoprotection, the ability to accumulate essential nutrients, and the

10 ability to continue to regulate transpiration in the presence of high concentrations of Na^+ and

11 Cl^- (Deinlein et al., 2014; Flowers & Colmer, 2008; Munns & Tester, 2008; Rozema &

12 Flowers, 2008; Shabala, 2013). Research has unveiled the complex, physiological, molecular,

13 and genetic background of these adaptations [e.g. (Ashraf & Foolad, 2013; Munns, 2005;

14 Munns & Tester, 2008; Shavrukov, 2012)]. There are more than 1,500 species of halophytes

15 (Aronson, 1989) and salt tolerance is widely distributed across the plant phylogeny, with

16 multiple independent origins (Flowers et al., 1977; Saslis-Lagoudakis et al., 2014). However

17 some plant groups, such as Caryophyllales and Alismatales, contain more halophytes than

18 others (Flowers et al., 2010; Saslis-Lagoudakis et al., 2014). At a lower hierarchical level, salt

19 tolerance has also evolved multiple times independently. For example, it has evolved over 70

20 times in the grass family alone, and is phylogenetically non-random, i.e. some clades are

21 more likely than others to contain salt tolerant species (Bennett et al., 2013).

22

23   It has been suggested that tolerance mechanisms and physiological responses to

24 salinity are shared with other types of environmental stresses, such as aridity, flooding, and

25 frost (Munns & Tester, 2008; Rozema & Schat, 2013; Tuteja, 2007). For example, a recent

study found that salt tolerance in grasses evolves more frequently in C_4 than C_3 lineages, demonstrating a close association in the evolution of C_4 photosynthesis and salt tolerance in these lineages (Bromham & Bennett, 2014). This type of correlations may provide one possible explanation for the repeated evolution of salt tolerance. The stress resistance syndrome hypothesis (Chapin et al., 1993) states that there may be a suite of stress-related traits that allow plants to survive in a variety of stressful environments. Therefore, the presence of "enablers" in some lineages can facilitate the evolution of multiple stress resistance within those lineages (Edwards & Donoghue, 2013). This suggests that traits related to tolerance to one type of stress can facilitate the evolution of another type of stress-resistance. For example, salt tolerance, succulence and C_4 photosynthesis are associated in chenopods (Kadereit et al., 2012) and occupation of bare environments served as an "enabler" to adaptation to harsh elemental soils in the Brassicaceae (Cacho & Strauss, 2014). Therefore, by studying these ecophysiological traits in a phylogenetic context, we can investigate macroevolutionary patterns of ecophysiological evolution (Ackerly et al., 2000), and explore the correlation between different ecophysiological strategies (Niinemets & Valladares, 2006).

A correlation of this kind has been suggested between salt and alkaline tolerance (Bromham et al., 2013; Bui, 2013; Bui et al., 2014). Alkalinity (high soil pH) often co-occurs with salinity (high soil NaCl concentrations) in the landscape: many saline soils are also alkaline due to the presence of sodium carbonates (Rengasamy, 2010). Therefore, it is possible that lineages occupying these environments have had to evolve strategies to cope with both alkalinity and salt-stress (Bui, 2013). Like salinity, alkalinity exacerbates water loss, interfering with stomatal closure due to the accumulation of sodium ions (Bernstein, 1975). Soils of high pH often have poor structure, affecting their hydraulic conductivity and

1   the plants' water uptake, and causing hypoxia in the root zone (Bernstein, 1975). Both these

2   factors affect water use efficiency, which is also one of the major stresses for plants in saline

3   environments. Plants equipped to deal with salinity and alkalinity employ osmotic

4   adjustments (Farrell et al., 1996; Yang et al., 2007; Yang et al., 2008) that are not found in

5   plants without tolerance to either stresses (Chen et al., 2011; Liu et al., 2010), and which

6   make tolerant plants naturally resistant to water stress (García & Mendoza, 2014). Further,

7   both salinity and alkalinity affect photosynthesis and metabolism through a range of

8   physiological and molecular processes (Nishiuchi et al., 2010; Yang et al., 2008). It is

9   possible, therefore, that because of the shared challenges, salt and alkaline tolerance have

10   evolved in closely related lineages which possess traits enabling the evolution of mechanisms

11   of tolerance to either stress.

12

13   One of the main constraints in exploring large-scale patterns in salt and alkaline

14   tolerance is the lack of exhaustive published lists of halophytes and particularly alkaline-

15   tolerant species. Because field and laboratory observations of plant species' tolerance to

16   salinity and alkalinity tend to focus on particular species, lists of known halophytes are likely

17   to be incomplete, and there are no comprehensive lists of alkaline tolerant species. An

18   alternative approach to generating such lists is to predict plant species that are tolerant to

19   these stresses based on their geographical distributions. In the last two decades, inferring

20   species' environmental niche preferences from their natural distributions and environmental

21   GIS data layers has become commonplace in studies of ecology and evolution (Guisan &

22   Thuiller, 2005; Kozak et al., 2008; Warren et al., 2008). By combining distribution data with

23   geochemical observations, we can infer salinity and alkalinity conditions to which species are

24   exposed in their natural distributions. Although microbial studies have combined

25   geochemical data with phylogenetic metrics (Costa et al., 2009; Macur et al., 2004;

1 Reysenbach & Shock, 2002), geochemical modelling has been largely overlooked in studies

2 of macroecology and macroevolution. However, a recent phylogenetic study of Australian

3 *Acacia* species used geochemical modelling to investigate evolutionary patters of salinity and

4 alkalinity tolerance (Bui et al., 2014).

5

6   The aims of this study were twofold: i) to evaluate the performance of geochemical

7 modelling using species occurrence data, to identify species' tolerance to salinity and

8 alkalinity, and ii) to investigate the correlation between salt and alkaline tolerance. We use

9 Australian grasses (Poaceae) as a test case, because they are a group with a continent-wide

10 distribution, occupying a wide range of environmental conditions, including arid, saline and

11 sodic environments. Our dataset included distribution data for 1,387 species of mainland

12 Australian grasses, of which 141 are known halophytes.

13

14 **Materials and Methods**

15   We investigated whether we could predict species' salt and alkaline tolerance based

16 on species distribution modelling. To do that, we used geochemical modelling to generate

17 species' descriptors for electrical conductivity (EC) and pH at their natural distributions. We

18 evaluated the prediction of salt-tolerant species based on prior knowledge of salt tolerance in

19 Australian grasses. Subsequently, we tested for the correlation between salt and alkalinity

20 tolerance, and we explored if spatial patterns can explain this association.

21

22   In the literature, salinity and alkalinity tolerance are often characterised based on EC

23 and pH soil values, respectively. For example, soils with EC over 4,000 $\mu S\ m^{-1}$ are

24 characterised as saline (United States Salinity Laboratory Staff, 1969) and plants tolerating

1   8,000 µS m^{-1} or over are considered halophytes (Aronson, 1989). Similarly, soil pH of 7 or

2   higher is alkaline and most plants prefer pH 5.5–6.5 (Islam et al., 1980). In this study, we do

3   no apply a threshold of EC or pH to characterise soils as saline or alkaline. Instead, we

4   perform a comparative analysis of EC and pH conditions to which Australian grasses are

5   exposed.

6

7   ***Predicting salt and alkalinity tolerance from species distribution modelling***

8   *Predicting species salt and alkalinity tolerance from occurrence data*

9   Because there are no exhaustive databases that describe tolerances of all Australian

10   grasses to salinity and alkalinity, in order to estimate these tolerances we employed an

11   approach based on species' distributions. Our approach assumes that conditions of salinity

12   and alkalinity at localities at which species are found naturally reflect their levels of tolerance

13   to these conditions. Although factors other than tolerance affect species' distributions, such as

14   interspecific competition, we can expect intrinsic tolerance to be correlated with realised

15   tolerances. Therefore, it is possible to describe species' tolerances if we know: i) species'

16   distributions and, ii) levels of salinity and alkalinity in these distributions. To generate

17   species' distributions, we extracted occurrence data from the Atlas of Living Australia

18   (ALA), a continent-wide dataset that contains approximately 45 million occurrence records

19   for Australian biodiversity. There are 1,387 grass species found in mainland Australia

20   (excluding Tasmania and other islands). Australian grass species are recorded from 354,913

21   points with unique geographic coordinates in the Atlas of Living Australia. We extracted all

22   unique occurrence points for each species and we consider the distribution of each species to

23   be the compilation of all the points at which it is reported.

24

1   In order to infer soil pH and electrical conductivity (EC) at the localities where grass

2   species were reported, we accessed data from the National Geochemical Survey of Australia.

3   This dataset reports the pH and EC on 1:5 soil:water extracts from bulk samples at 1,315

4   georeferenced point measurements across the continent, with an average sample density of 1

5   site/5,500 km^2 (de Caritat & Cooper, 2011). We retrieved indications of EC and pH from the

6   dataset and performed the analyses described below for subsoil (60-80 cm below the surface).

7   Subsoil indications of EC and pH are more likely to reflect tolerance to salinity and alkalinity

8   than shallower samples, as root tips - generally found deeper in the soil - are more highly

9   sensitive to geochemistry than the rest of the root (Shabala, 2013).

10

11   From this dataset of subsoil EC and pH indications, we estimated EC and pH at each

12   locality with a reported grass occurrence using Geostatistics in *geoR* (Diggle & Ribeiro,

13   2007). Geostatistics are techniques for mapping of surfaces from limited sample data and the

14   estimation of values at unsampled locations in two steps (Clark & Harper, 2000): First, a

15   semi-variogram was constructed to establish the predictability of values from place to place

16   in the study area. The semi-variogram modelled the difference between a value at one

17   location and the value at another according to the distance and between them. Secondly,

18   "kriging" was used to estimate values at unsampled locations. The basic technique of

19   ordinary kriging that we used here used a weighted average of neighbouring samples to

20   estimate the value at an unsampled location. Weights were optimised using the semi-

21   variogram model, given the distance and directional relationships between sampled and

22   unsampled locations. We used the ordinary kriging variance as an estimate of error associated

23   with each prediction (Diggle & Ribeiro, 2007). With this approach, we produced a

24   compilation of EC and pH predictions for each species; given each individual prediction

25   corresponds to an estimate for each location at which the species is recorded. This gives a

1   range of predicted EC and pH values for each species, and from this range we recorded the

2   median and upper quartile (UQ) values. Therefore, for each species, we used four measures

3   to describe soil salinity and alkalinity across its distribution: two describing EC (median and

4   UQ values) and two describing pH (again, median and UQ values). Median values provide

5   species' central tendency with respect to environmental conditions (EC and pH) in their

6   distributions, while UQ values represent more extreme salinity and alkalinity conditions that

7   species encounter within their geographic ranges.

8

9   *Evaluating prediction of halophytes*

10   An ideal way to evaluate how well the geochemical modelling approach performed in

11   predicting species' salinity and alkalinity tolerance, would be to test species' tolerances

12   experimentally, as well as to take EC and pH measurements at localities where species occur

13   naturally, covering each species range, and then compare those measurements to our

14   predictions. However, to generate this data, even for one single species, would require

15   considerable amount of time and effort. An alternative way to evaluate the performance of

16   the geochemical modelling is using data that is already available. Although we do not have

17   prior knowledge of alkaline-tolerant species, we have lists of halophytes. These lists might be

18   incomplete, but they are likely to be accurate in the species that are included, as they are

19   based on expert judgment and experimental data. Because halophytes are able to grow in

20   conditions of high salinity, the predicted EC for taxa known to be halophytes should be

21   higher than that for non-halophytes.

22

23   Here, we asked whether known halophytes have been reported to occur at higher

24   predicted EC than non salt-tolerant species. First, we extracted the species names of known

25   Australian grass halophytes from a recent study (Bennett et al., 2013), which identified 141

1   Australian grasses as halophytes (Supplementary Table 1). Then, we applied a parametric

2   Welch two sample t-test to test if predicted EC values (median and UQ) of known halophytes

3   were significantly higher than the rest of the species in our dataset.

4

5   Further, we performed the same analysis (Welch two sample t-test) at the genus level,

6   to ask if genera containing halophytes occur in conditions of high predicted EC. There are

7   234 Australian grass genera in total, 71 of which include at least one known halophyte. We

8   calculated median and upper quartile soil EC values for each genus, based on the

9   observations for all species within that genus. Further, we used a phylogeny-corrected two

10   sample t-test. We estimated the phylogenetic correlation matrix among genera using two

11   phylogenies. One is a well-sampled genus-level topology of Poaceae that includes over 800

12   genera (Bouchenak-Khelladi et al., 2010). This tree included 226 of the 234 Australian

13   genera and 70 of the 71 genera with known haplotypes. We computed the branch lengths of

14   the topology using the method by Grafen (1989), which gives each node on the tree a

15   'height', corresponding to the number of leaves of the subtree minus one. Each height was

16   scaled so that root height is 1, and then raised at power "rho" (Grafen, 1989). Branch lengths

17   were then calculated as the difference between height of lower and upper nodes. The other

18   phylogeny was a smaller, time-calibrated molecular phylogenetic tree with 298 out of

19   approximately 800 genera of Poaceae (Bouchenak-Khelladi et al., 2010). This tree included

20   146 of 234 Australian genera and 56 of 71 Australian genera with known haplotypes. We

21   performed the analysis using this tree because, although taxon sampling was limited, it was

22   time-calibrated, and we wanted to ensure that the absence of branch lengths in the larger

23   phylogenetic tree did not affect our results. We accounted for phylogenetic relatedness in a

24   two sample t-test using Generalized Least Squares (GLS) approach. GLS is a generalised

25   approach for estimating parameters in a linear regression model where observations are not

1   homoscedastic or independent from each other (Martins & Hansen, 1997). The phylogenetic

2   relatedness was accounted for by correcting the covariance matrix among observations

3   according to their phylogenetic relatedness (Martins & Hansen, 1997).

4

5   The parametric test compared predicted EC values for halophytic taxa to predicted EC

6   values of the rest of the taxa, and evaluated whether halophytic taxa had higher predicted EC

7   than non-halophytic taxa. Because salt tolerance is not randomly distributed in the grass

8   phylogeny (Bennett et al., 2013), by accounting for phylogenetic relatedness, the

9   phylogenetic test ensured that if a relationship was recovered, it was beyond that expected

10   from phylogeny.

11

12   ***Testing the correlation between salt and alkalinity tolerance***

13   *Correlation of taxa occurring in high predicted salinity and alkalinity*

14   We asked whether the taxa found in conditions of high predicted salinity also tended

15   to be found in conditions of high predicted alkalinity. Similar to the previous section, we first

16   calculated the median and UQ EC and pH values for each taxon. We performed this analysis

17   at the species level, testing the correlation between species' median or UQ EC values and

18   species' median or UQ pH values, using the parametric Pearson's product-moment

19   correlation. The same analysis was performed at the genus level, along with a phylogenetic

20   reduced major axis (RMA) regression (Ives et al., 2007), using the two phylogenies described

21   above to estimate the phylogenetic correlation matrix. RMA regression is a type II regression

22   that does not assume causal directionality between values of salinity and alkalinity. The

23   phylogenetic relatedness is accounted for by a similar approach as in GLS (Ives et al., 2007;

24   Martins & Hansen, 1997). Although the parametric test evaluates the correlation between

predicted EC and pH for taxa, the phylogenetic test evaluates whether this correlation is because of covariation due to shared ancestry among taxa.

Geographical correlation of salinity and alkalinity

We wanted to tease apart whether any association between predicted salinity and alkalinity values was due to geographical correlation between soil EC and pH. First, to assess the degree to which salinity and alkalinity overlapped on the landscape in areas where Australian grasses are found, we fitted a linear model between predicted values of EC and pH for all occurrence points where Australian grasses were reported. If at localities where predicted EC was high, predicted pH was also high (and vice versa), then species exposed to high salinity were also exposed to high alkalinity (and vice versa).

Second, we tested for the correlation between predicted salinity and alkalinity only for known halophytes, using a parametric Pearson's product-moment correlation. We also tested this relationship at the genus level, only for genera that contain known halophytes, with the parametric Pearson's product-moment correlation, and a phylogenetic reduced major axis (RMA) regression (Ives et al., 2007), using the two phylogenies to estimate the phylogenetic correlation matrix. If salt and alkalinity-tolerance were functionally associated but conditions of salinity and alkalinity were not geographically associated, then salt-tolerant taxa could be found in conditions of both low and high alkalinity. Under these conditions, we would expect a weaker correlation between predicted EC and pH values in salt-tolerant than non salt-tolerant taxa. If salt and alkalinity-tolerance were functionally associated and conditions of salinity and alkalinity were geographically associated, we would expect a stronger correlation between predicted EC values and pH values in in salt-tolerant than non salt-tolerant taxa.

1

2   All statistical analyses used log-transformed EC values for normality and were

3   implemented in R (R Core Team, 2014), with Grafen's computation of branch lengths

4   (Grafen, 1989) using the 'compute.brlen' function in 'ape' package (Paradis et al., 2004), the

5   phylogeny-corrected t-test using the 'gls' function in 'nlme' package (Pinheiro et al., 2014),

6   and the phylogenetic RMA regression using the 'phyl.RMA' function in 'phytools' package

7   (Revell, 2012).

8

9   **Results**

10 ***Predicting salt and alkalinity tolerance from species distribution modelling***

11 *Predicting species salt and alkalinity tolerance from occurrence data*

12   Predicted soil EC for all occurrence points where Australian grasses are found ranged

13 between 0.01 and 10.53 dS m^{-1} and predicted pH ranged from 4.87 to 9.05. The average

14 standard error (as estimated with kriging variance) for predictions across all reported

15 localities was 2.06 dS m^{-1} for EC and 0.93 for pH.

16

17 *Evaluating prediction of halophytes*

18   Our results (Table 1) show that halophytic species are not found in significantly

19 higher predicted salinity than non salt-tolerant species. However, both analyses (parametric

20 and phylogeny-corrected) at the genus level, considering both median and UQ predicted EC,

21 suggest that genera with known halophytes are found in significantly higher predicted soil EC

22 than genera that do not include known halophytes. Although significantly positive, the

23 absolute difference in EC values between genera with and without known halophytes is

1 small. The predicted EC values for genera with known halophytes only explains about 5%

2 variation of the total of EC values in our dataset (R^2 in Table 1).

3

4 ***Testing the correlation between salt and alkalinity tolerance***

5 *Correlation of taxa occurring in high predicted salinity and alkalinity*

6   Our results indicate that species found in conditions of high predicted salinity also

7 tend to be found in conditions of high predicted alkalinity. This is true when considering

8 species' median and UQ EC and pH (Table 1). The same result is found at the genus level,

9 including when accounting for phylogenetic relatedness (Table 2).

10

11 *Geographical correlation of salinity and alkalinity*

12   The Pearson correlation coefficient (r) can range between -1 (total negative

13 correlation) and 1 (total positive correlation), with 0 denoting no correlation. The value we

14 recovered for the correlation between predicted EC and pH at localities where species were

15 found is very close to 0 (0.0003), suggesting this correlation is extremely weak. Although we

16 found a significant effect ($p < 0.001$), this could be due to a weak relationship in a large

17 amount of data points (N = 354,913). We found a stronger correlation between predicted EC

18 values and pH values for salt-tolerant than for non salt-tolerant taxa, both at the species and

19 genus level (Table 1, Table 2, Figure 1).

20

1 **Discussion**

2 *Predicting salt and alkalinity tolerance from species distribution modelling*

3   The motivation for this study was to explore a possible correlation between salt and

4 alkaline tolerance (Bromham et al., 2013; Bui, 2013; Bui et al., 2014), using Australian

5 grasses as an example. We used a geochemical modelling approach to predict the conditions

6 of salinity and alkalinity in which species occur in their natural distributions (Bui et al.,

7 2014). There are some limitations to this approach. First, our EC predictions were based on

8 measurements in dilute (1:5) solutions compared to the salt concentrations that plants would

9 encounter in saline soils. Predicted electrical conductivity (EC) across localities where grass

10 species were found ranged from 0.01 to 10.53 dS m^{-1}, and halophytes are often described as

11 species that complete their life-cycles in soils of 8 dS m^{-1} and above (Aronson, 1989). Very

12 few localities in our dataset were found above that threshold and only four known halophytes

13 are found in these localities. Nevertheless, our geochemical modelling approach was not used

14 to predict species' absolute tolerances, but relative tolerances that can be used in a

15 comparative framework. Second, it is possible that the geochemical modelling does not

16 accurately capture variation in salinity at the scale that is relevant to ecophysiology. Salinity

17 varies on a micro-scale, depending on many factors, such as climate, lithology, topography,

18 and vegetation (Bui, 2013). Plant distributions can be determined by the distribution of

19 salinity at that scale, but that will not necessarily be picked up by these landscape-level

20 estimates.

21

22   Because of these possible restrictions, we wanted to evaluate the relevance of our

23 geochemical predictions to plant salt tolerance. To do so, we compared predicted salinity

24 values for known halophytic taxa to the rest of the taxa in our dataset. Using a parametric

25 Welch two sample t-test, we found that predicted EC for known halophytes is not

1   significantly higher than that for non-halophytes. Nevertheless, when testing this relationship

2   at the genus level, we found that genera with known halophytes have significantly higher

3   predicted soil EC than genera that do not include known halophytes, using a parametric and a

4   phylogeny-corrected approach. This is likely due to the fact that the list of known halophytes

5   in Australian grasses is much more incomplete than the list of genera with known halophytes.

6   Treating unrecognised halophytes that have high predicted EC values as non-halophytes

7   could contribute to the smaller effect size (R^2 value) in the species-level analyses compared to

8   the genus-level ones, as we show in Tables 1 and 2. We explored two different values to

9   represent predicted EC for each taxon: median and UQ. Our results show that UQ,

10 representing the more extreme values of EC, is better at predicting clades with halophytes,

11 because the effect size (R) is always larger for UQ values than for median values (Table 1,

12 Table 2). It is problematic that some known halophytes are not found in high predicted EC

13 (Figure 1), suggesting that our geochemical approach does not identify salt tolerance

14 successfully. However, our predicted EC values have the potential to identify groups of

15 possible halophytes. The main goal of this study was to investigate the correlation between

16 salt and alkaline tolerance. Therefore, as mentioned above, we aimed at generating relative –

17 rather than absolute - tolerances that can be analysed comparatively for all taxa in the dataset.

18

19 ***Patterns of correlation between salt and alkalinity tolerance***

20   Previous studies have found correlations between different types of ecophysiological

21 strategies related to environmental stress tolerance, particularly to water use efficiency. For

22 example, salt-tolerant grasses have evolved more frequently in lineages with C_4

23 photosynthesis, potentially because these lineages can control water loss better than C_3

24 lineages, giving them an advantage to adapt to arid saline environments (Bromham &

25 Bennett, 2014). A correlation was found between salt tolerance, succulence and C_4

photosynthesis in chenopods (Kadereit et al., 2012), and a similar evolutionary correlation

has been found between CAM photosynthesis and succulence (Ogburn & Edwards, 2010), as

well as for occupation of bare environments and to adaptation to harsh elemental soils in the

Brassicaceae (Cacho & Strauss, 2014).

Our results suggest that salt and alkaline tolerance are associated: we found that

species found in conditions of high predicted salinity tend to be found in conditions of high

predicted alkalinity (Table 1). This relationship was also recovered at the genus level,

including when correcting for phylogenetic relationships (Table 2). This is in agreement with

the recent finding that salt and alkaline tolerance are also linked on the phylogeny of

Australian *Acacia* (Bui et al., 2014). One possible explanation for the association between

taxa in high predicted salinity and alkalinity is the presence of "enablers" in some lineages

that can facilitate the evolution of multiple stress resistance within those lineages (Edwards &

Donoghue, 2013). It could be that some lineages have traits that provide "stepping stones" to

developing both salt and alkaline tolerance: that is, lineages may have traits that do not in

themselves confer salt tolerance but make it easier for those lineages to evolve tolerance of

saline or alkaline conditions.

However, the correlation we find could also be driven by the overlap of salinity and

alkalinity in the landscape (Rengasamy, 2010). We assessed the degree to which predicted

salinity and alkalinity correlated in localities where Australian grasses are reported. The

correlation between EC and pH at species' localities is significant, but it does not explain

much of the variation in our data. Therefore, species exposed to high predicted EC are not

necessarily also exposed to high predicted pH at the same localities. For example, as shown

in Supplementary Figure 1, the highest predicted EC values are found in both predicted

1   alkaline and acidic soils, and the localities with the highest predicted pH values have low to

2   relatively high predicted EC. Nonetheless, predicted EC values and pH values are more

3   strongly associated for salt-tolerant than for non salt-tolerant taxa (Table 1, Table 2, Figure

4   1). Therefore, we cannot discount the effect of the overlap of salinity and alkalinity in the

5   landscape in shaping the pattern of correlation we found here. Further research is needed to

6   evaluate how much this overlap contributes to the recovered pattern.

7

8   As we have demonstrated, geochemical modelling predictions may provide useful

9   starting points for further investigations of macroevolutionary patterns between salt and

10   alkaline tolerance. However, we did not investigate soil ion chemistry across localities where

11   Australian grasses are found, which affects the correlation between salinity and alkalinity in

12   the soil. For example, soil pH between 7 and 10 mainly reflects anions in solution and when

13   neutral salts such as NaCl or Na_2SO_4 are in solution, sulphate and chloride anions dominate

14   and pH is between 6 and 8 (Rengasamy, 2010). When bicarbonate and carbonate ions

15   dominate, pH rises above 8. At pH above 9, carbonate ions are dominant. Alkaline soils

16   without salt can have pH above 9 but when NaCl is present, pH is lower. However for pH

17   measured in 1:5 soil:water as estimated here, the decrease in pH associated with the presence

18   of NaCl will be diminished by dilution. Also, not all alkaline soils are toxic for plants. For

19   instance, although calcareous soils - abundant in Australia - can be an edaphic barriers to

20   plant radiation [e.g. Nullarbor Plain (Crisp & Cook, 2007)], no toxicity has been observed in

21   lime (CaCO3) dominant soils: although they are alkaline, the solubility of $CaCO_3$ is low and

22   the carbonate concentration is usually around 1 mmolc/L. Given this, we believe more

23   phylogenetic analyses incorporating more complete soil chemistry, as well as testing soil

24   toxicity across sites (Cacho & Strauss, 2014), can lead to more detailed explanations of our

25   reported correlations between salinity and alkalinity for grasses. Further, future investigations

1   could focus on specific traits that might be shared between salinity and alkalinity-tolerances.

2   For example, similar osmotic responses to salinity and alkalinity (Chen et al., 2011; Liu et al.,

3   2010) suggest that some shared mechanisms might be involved in managing water use

4   efficiency under salt and alkaline tolerance. These mechanisms can be investigated

5   experimentally, but a comparative phylogenetic framework may also be useful. For example,

6   species' geochemical predictions can be analysed in a comparative framework that can reveal

7   the degree to which phylogenetic relatedness or spatial autocorrelation can explain the

8   variation in these datasets (Freckleton & Jetz, 2009).

9

10 ***Conclusions***

11   In this study, we used distribution data for Australian grasses combined with

12 geochemical modelling to predict the range of values of soil salinity and alkalinity to which

13 species are exposed. The aim of this study was to evaluate the use of geochemical modelling

14 in identifying taxa that can tolerate conditions of high salinity and alkalinity. Therefore, our

15 approach was not used to predict species' absolute tolerances, but relative tolerances that can

16 be used in a comparative framework. We find that our geochemical predictions, despite their

17 limitations, can identify known halophytic taxa as present in conditions of relatively high

18 salinity. We also found that grass taxa found in areas of high predicted salinity also tend to be

19 found in conditions of high predicted alkalinity. This pattern could suggest a correlation

20 between salt and alkalinity-tolerance, for example due to the presence of enabling traits that

21 promote the evolution of salinity and alkalinity tolerance. Our approach provides a valuable

22 test of the use of geochemical modelling to predicting abiotic stress tolerances, beyond those

23 related to temperature and precipitation. Further investigations could consider the

24 phylogenetic distribution of specific traits involved in these ecophysiological strategies,

1   ideally by incorporating more comprehensive and finer scale information on variation of

2   geochemistry in the landscape.

3

4   **Acknowledgments**

5   We thank Dan Warren for helpful comments during this study.

6

7   **Funding information**

8   This work was supported by the Australian Research Council.

Literature cited

Ackerly, D. D., Dudley, S. A., Sultan, S. E., Schmitt, J., Coleman, J. S., Linder, C. R., Sandquist, D. R., Geber, M. A., Evans, A. S., Dawson, T. E. & Lechowicz, M. J. (2000) The evolution of plant ecophysiological traits: recent advances and future directions. *BioScience 50*, 979-995.

Atlas of Living Australia website at http://www.ala.org.au. Accessed 15 November 2013 [database on the Internet].

Aronson, J. (1989) *HALOPH a data base of salt tolerant plants of the world*. Arizona: Office of Arid Land Studies, University of Arizona.

Ashraf, M. & Foolad, M. R. (2013) Crop breeding for salt tolerance in the era of molecular markers and marker-assisted selection. *Plant Breeding 132*, 10-20.

Bennett, T. H., Flowers, T. J. & Bromham, L. (2013) Repeated evolution of salt-tolerance in grasses. *Biology Letters 9*.

Bernstein, L. (1975) Effects of salinity and sodicity on plant growth. *Annual Review of Phytopathology 13*, 295-312.

Bouchenak-Khelladi, Y., Verboom, G. A., Savolainen, V. & Hodkinson, T. R. (2010) Biogeography of the grasses (Poaceae): a phylogenetic approach to reveal evolutionary history in geographical space and geological time. *Botanical Journal of the Linnean Society 162*, 543-557.

Bromham, L. & Bennett, T. H. (2014) Salt tolerance evolves more frequently in C4 grass lineages. *Journal of Evolutionary Biology 27*, 653-659.

Bromham, L., Saslis-Lagoudakis, C. H., Bennett, T. H. & Flowers, T. J. (2013) Soil alkalinity and salt tolerance: adapting to multiple stresses. *Biology Letters 9*.

Bui, E. N. (2013) Soil salinity: A neglected factor in plant ecology and biogeography. *Journal of Arid Environments 92*, 14-25.

Bui, E. N. (2013) Possible role of soil alkalinity in plant breeding for salttolerance. *Biology Letters 9*.

Bui, E. N., Thornhill, A. & Miller, J. T. (2014) Salt- and alkaline-tolerance are linked in Acacia. *Biology Letters 10*.

Cacho, N. I. & Strauss, S. Y. (2014) Occupation of bare habitats, an evolutionary precursor to soil specialization in plants. *Proceedings of the National Academy of Sciences 111*, 15132-15137.

Chapin, F. S., Autumn, K. & Pugnaire, F. (1993) Evolution of suites of traits in response to environmental stress. *The American Naturalist 142*, S78-S92.

Chen, W., Feng, C., Guo, W., Shi, D. & Yang, C. (2011) Comparative effects of osmotic-, salt- and alkali stress on growth, photosynthesis, and osmotic adjustment of cotton plants. *Photosynthetica 49*, 417-425.

Clark, I. & Harper, W. V. (2000) *Practical geostatistics*. Columbus, OH: Ecosse North American LLC.

Costa, K. C., Navarro, J. B., Shock, E. L., Zhang, C. L., Soukup, D. & Hedlund, B. P. (2009) Microbiology and geochemistry of great boiling and mud hot springs in the United States Great Basin. *Extremophiles 13*, 447-459.

Crisp, M. D. & Cook, L. G. (2007) A congruent molecular signature of vicariance across multiple plant lineages. *Molecular Phylogenetics and Evolution 43*, 1106-1117.

de Caritat, P. & Cooper, M. (2011) *National Geochemical Survey of Australia: The Geochemical Atlas of Australia. Record 2011/20*. Canberra: Geoscience Australia.

Deinlein, U., Stephan, A. B., Horie, T., Luo, W., Xu, G. & Schroeder, J. I. (2014) Plant salt-tolerance mechanisms. *Trends in Plant Science*.

Diggle, P. & Ribeiro, P. (2007) *Model-based geostatistics*: Springer.

Edwards, E. J. & Donoghue, M. J. (2013) Is it easy to move and easy to evolve? Evolutionary accessibility and adaptation. *Journal of Experimental Botany*.

Farrell, R., Bell, D., Akilan, K. & Marshall, J. (1996) Morphological and physiological comparisons of clonal lines of *Eucalyptus camaldulensis*. II. Responses to waterlogging/salinity and alkalinity. *Functional Plant Biology 23*, 509-518.

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

Flowers, T. J., Galal, H. K. & Bromham, L. (2010) Evolution of halophytes: multiple origins of salt tolerance in land plants. *Functional Plant Biology 37*, 604-612.

Flowers, T. J., Troke, P. F. & Yeo, A. R. (1977) The mechanism of salt tolerance in halophytes. *Annual Review of Plant Physiology 28*, 89-121.

Freckleton, R. P. & Jetz, W. (2009) Space versus phylogeny: disentangling phylogenetic and spatial signals in comparative data. *Proceedings of the Royal Society B: Biological Sciences 276*, 21-30.

García, I. & Mendoza, R. (2014) *Lotus tenuis* seedlings subjected to drought or waterlogging in a saline sodic soil. *Environmental and Experimental Botany 98*, 47-55.

Grafen, A. (1989) The phylogenetic regression. *Philosophical Transactions of the Royal Society of London B 326*, 119-157.

Guisan, A. & Thuiller, W. (2005) Predicting species distribution: offering more than simple habitat models. *Ecology Letters 8*, 993-1009.

Islam, A. K. M. S., Edwards, D. G. & Asher, C. J. (1980) pH optima for crop growth. *Plant and Soil 54*, 339-357.

Ives, A. R., Midford, P. E. & Garland, T., Jr. (2007) Within-species variation and measurement error in phylogenetic comparative methods. *Systematic Biology 56*, 252-270.

Kadereit, G., Ackerly, D. & Pirie, M. D. (2012) A broader model for C4 photosynthesis evolution in plants inferred from the goosefoot family (Chenopodiaceae s.s.). *Proceedings of the Royal Society B: Biological Sciences 279*, 3304-3311.

Kozak, K. H., Graham, C. H. & Wiens, J. J. (2008) Integrating GIS-based environmental data into evolutionary biology. *Trends in Ecology & Evolution 23*, 141-148.

Liu, J., Guo, W. Q. & Shi, D. C. (2010) Seed germination, seedling survival, and physiological response of sunflowers under saline and alkaline conditions. *Photosynthetica 48*, 278-286.

Macur, R. E., Langner, H. W., Kocar, B. D. & Inskeep, W. P. (2004) Linking geochemical processes with microbial community analysis: successional dynamics in an arsenic-rich, acid-sulphate-chloride geothermal spring. *Geobiology 2*, 163-177.

Martins, E. P. & Hansen, T. F. (1997) Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *The American Naturalist 149*, 646-667.

Munns, R. (2005) Genes and salt tolerance: bringing them together. *New Phytologist 167*, 645-663.

Munns, R. & Tester, M. (2008) Mechanisms of salinity tolerance. *Annual Review of Plant Biology 59*, 651-681.

Niinemets, Ü. & Valladares, F. (2006) Tolerance to shade, drought, and waterlogging of temperate Northern Hemisphere trees and shrubs. *Ecological Monographs 76*, 521-547.

Nishiuchi, S., Fujihara, K., Liu, S. & Takano, T. (2010) Analysis of expressed sequence tags from a NaHCO3-treated alkali-tolerant plant, *Chloris virgata*. *Plant Physiology and Biochemistry 48*, 247-255.

Ogburn, R. M. & Edwards, E. J. (2010) The ecological water-use strategies of succulent plants. *Advances in Botanical Research 55*, 179-225.

Paradis, E., Claude, J. & Strimmer, K. (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics 20*, 289-290.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-117. 2014.

R Core Team. R: A Language and Environment for Statistical Computing. 2014.

Rengasamy, P. (2010) Soil processes affecting crop production in salt-affected soils. *Functional Plant Biology 37*, 613-620.

Revell, L. J. (2012) phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution 3*, 217-223.

Reysenbach, A.-L. & Shock, E. (2002) Merging Genomes with Geochemistry in Hydrothermal Ecosystems. *Science 296*, 1077-1082.

Rozema, J. & Flowers, T. (2008) Crops for a salinized world. *Science 322*, 1478-1480.

Rozema, J. & Schat, H. (2013) Salt tolerance of halophytes, research questions reviewed in the perspective of saline agriculture. *Environmental and Experimental Botany 92*, 83-95.

Saslis-Lagoudakis, C. H., Moray, C. & Bromham, L. (2014) Evolution of salt tolerance in angiosperms: A phylogenetic approach. In: Rajakaruna, N, Boyd, B, Harris, T (Eds.), *Plant Ecology and Evolution in Harsh Environments*, (pp. 77-95)

Shabala, S. (2013) Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops. *Annals of Botany 112*, 1209-1221.

Shavrukov, Y. (2012) Salt stress or salt shock: which genes are we studying? *Journal of Experimental Botany 64*, 119-127.

Tuteja, N. (2007) Mechanisms of high salinity tolerance in plants., *Osmosensing and osmosignaling*, (pp. 419-438). San Diego, CA: Elsevier Academic Press.

United States Salinity Laboratory Staff. (1969) *Diagnosis and improvement of saline and alkali soils*. Washington, DC: United States Department of Agriculture.

Warren, D. L., Glor, R. E. & Turelli, M. (2008) Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. *Evolution 62*, 2868-2883.

Yang, C., Chong, J., Li, C., Kim, C., Shi, D. & Wang, D. (2007) Osmotic adjustment and ion balance traits of an alkali resistant halophyte *Kochia sieversiana* during adaptation to salt and alkali conditions. *Plant and Soil 294*, 263-276.

Yang, C., Shi, D. & Wang, D. (2008) Comparative effects of salt and alkali stresses on growth, osmotic adjustment and ionic balance of an alkali-resistant halophyte *Suaeda glauca* (Bge.). *Plant Growth Regulation 56*, 179-190.

1 **Tables**

2

3 Table 1. Results of tests for the comparison of predicted EC values for known halophytes vs.

4 non salt-tolerant species, and for the correlation between salinity and alkalinity conditions in

5 Australian grass species. Alternative hypotheses are listed in the first column. The variable

6 tested (median or UQ) for species' salinity and/or alkalinity is given in the second column.

7 Each hypothesis was tested with parametric test. t-statistic and R^2 values are reported for each

8 test. Statistics significant at 0.05 level are marked with an asterisk; significant at 0.005 level

9 are marked with a double asterisk. Significant statistics support the alternative hypotheses.

10

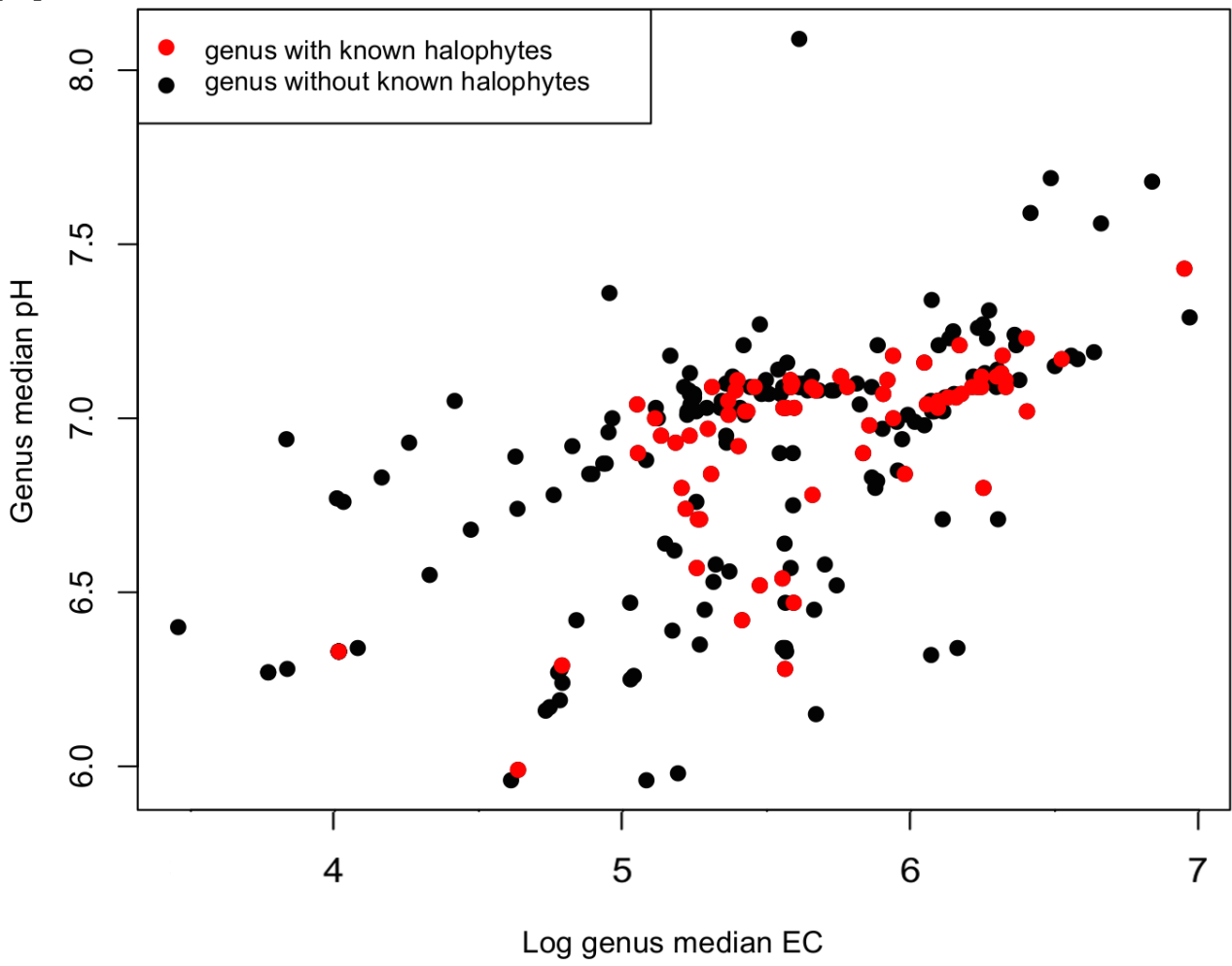| Alternative hypothesis	Variable	Parametric
Known halophytes are found in conditions of higher predicted salinity than non salt-tolerant species	Median	$t_{185}=0.54$ $R^2=0.00$
	Upper quartile	$t_{185}=1.14$ $R^2=0.01$
Species found in conditions of high predicted salinity also tend to be found in conditions of high predicted alkalinity	Median	$T_{1385}=29.63**$ $R^2=0.39$
	Upper quartile	$T_{1385}=35.96**$ $R^2=0.48$
Known halophytes found in conditions of high predicted salinity tend to be found in conditions of high predicted alkalinity	Median	$T_{139}=12.33**$ $R^2=0.52$
	Upper quartile	$T_{139}=17.88**$ $R^2=0.70$

11

1   Table 2. Results of tests for the comparison of predicted EC values for genera including

2   known halophytes vs. those not including halophytes, and for the correlation between salinity

3   and alkalinity conditions in Australian grass genera. Alternative hypotheses are listed in the

4   first column. The variable tested (median or UQ) for salinity and/or alkalinity of a given

5   taxon is given in the second column. Tests for each hypothesis include a parametric and two

6   phylogeny-corrected analyses. The phylogeny-corrected analyses were performed on a

7   complete genus-level phylogenetic tree of grasses (Complete column) and a smaller, time-

8   calibrated phylogenetic tree (Calibrated column) from a previous study (Bouchenak-Khelladi

9   et al., 2010). t-statistic and R^2 values are reported for each test. Statistics significant at 0.05

10   level are marked with an asterisk; significant at 0.005 level are marked with a double asterisk.

11   Significant statistics support the alternative hypotheses.

12

Alternative hypothesis	Variable	Parametric	Phylogeny-corrected	
			Complete	Calibrated
Genera with known halophytes are found in conditions of higher predicted salinity than genera without known halophytes	Median	$t_{186}=3.25**$ $R^2=0.04$	$t_{224}=3.03**$ $R^2=0.04$	$t_{144}=2.45*$ $R^2=0.04$
	Upper quartile	$t_{209}=3.89**$ $R^2=0.06$	$t_{144}=4.44**$ $R^2=0.08$	$t_{144}=2.46*$ $R^2=0.04$
Genera found in conditions of high predicted salinity tend to be found in conditions of high predicted alkalinity	Median	$t_{232}=11.18**$ $R^2=0.35$	$t_{198}=12.60**$ $R^2=0.28$	$t_{116}=9.02**$ $R^2=0.53$
	Upper quartile	$t_{232}=15.60**$ $R^2=0.51$	$t_{144}=16.68**$ $R^2=0.56$	$t_{109}=13.75**$ $R^2=0.70$
Genera with known halophytes found in conditions of high predicted salinity tend to be found in conditions of high predicted alkalinity	Median	$t_{69}=6.71**$ $R^2=0.40$	$t_{55}=3.96**$ $R^2=0.35$	$t_{45}=6.22**$ $R^2=0.62$
	Upper quartile	$t_{69}=9.05**$ $R^2=0.54$	$t_{55}=5.96**$ $R^2=0.56$	$t_{43}=10.33**$ $R^2=0.72$

Figure Legends

Figure 1. Correlation between predicted soil salinity and alkalinity for Australian grass genera. The predicted salinity and alkalinity of a given genus is measured as the median (A) and upper quartile (B) value of all predictions of electrical conductivity (EC) or pH, respectively, for all localities where species of that genus occur in mainland Australia. Black dots are genera that do not include any known halophytes and red dots are genera that include known halophytes.

A

B