

Department of **Biodiversity**, **Conservation and Attractions** 



# Seasonal ecophysiological variation and plant mineral nutrition of *Aluta quadrata*

## **Annual Report 2**

# March 2022 – March 2023

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## **Executive summary**

This annual report summarises the research conducted in Year 2 of the *Aluta quadrata* plant water use and niche characteristics research. Broadly, the second year focused on:

- 1. Continuing seasonal measurements in *Aluta quadrata* plants in sites that vary in modelled suitability and comparing sites with *Aluta quadrata* present and <u>absent</u> via the co-occurring, morphologically similar *Eremophila latrobei*;
- 2. Characterising plant mineral nutrition in high and low suitability sites; and
- 3. Describing water sourcing in plants along an elevation gradient.

Our ecophysiological monitoring demonstrates clear seasonal variation in gas exchange, chlorophyll performance and plant water stress, with peak and stress periods for ecophysiological functioning related to wetter periods (e.g., March – May) and pre-summer dry periods (e.g. October), respectively. There were moderate to strong statistical differences observed in ecophysiological traits among high and low suitability (inferred by probability of occurrence) sites—with high predicted suitability demonstrating increased ecophysiological performance with these differences associated with higher plant available water from rainfall in the surrounding niche. In addition, for both *A. quadrata* and *E. latrobei*, there were species-level differences in sites where *A. quadrata* are present and site-level differences where *A. quadrata* plants were <u>absent</u>, suggesting low confidence in *E. latrobei* plants as a model species for site selection. While our survey efforts have only covered two suitability sites for *A. quadrata*, further grounding of ecophysiological trait and predicted site suitability relationships, as well as understanding plant responses in relation to their local habitat positioning will be the next logical step for ongoing research.

Overall, soils were characterised as nutrient poor with variation found between sites. Plant nutrient status varied significantly between both sites and species. In *Aluta quadrata*, high suitability sites had soils higher pH and higher clay contents. High suitability sites also demonstrated higher plant magnesium and calcium percentages, as well as reduced plant and soil phosphorus concentrations.

To understand water sourcing in *A. quadrata* plants, we studied plant isotopic signatures along a suitability, as well as elevation gradient. We sampled plants, as well as soils in the direct vicinity of plant at approximately 300 mm depth, to determine isotopic signatures for  $\delta^2$ H,  $\delta^{18}$ O, and  $\delta^{13}$ C. Sample analysis is currently close to completion and will be summarised in an interim report in the third quarter of 2023.

Our final year of research will complete ecophysiological surveys with a specific focus of targeting a greater variation of suitability classes in the landscape for *A. quadrata*. By measuring more broadly across the landscape, we aim to capture greater variation in ecophysiological performance which will help with model refinements. In addition, as isotope studies suggest plants are sourcing water from atmospheric rainfall or from shallow-lying moisture reserves, understanding root depth of the species would further unravel plant-water sourcing dynamics and growth mechanisms in the soil. Root studies would also provide the opportunity to understand possible mycorrhizal associations that underpin plant-soil interactions of the species.

## Acknowledgement

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## Background

In response to a request from Rio Tinto Iron Ore (RTIO), Kings Park Science commenced research into the plant-water use and niche definition of *Aluta quadrata* in March 2021. *Aluta quadrata* is a threatened myrtaceous shrub native to the Pilbara region of Western Australia which grows in three distinct populations spanning a ~38.5km distribution along the southern fringe of the Hamersley Range (Byrne et al., 2016).

*Aluta quadrata* grows in habitat including steep rocky slopes, steep gorges, and gullies, with a preference for southern facing slopes of rugged topography in skeletal soils, including Brockman Iron Formation substrates (Byrne et al. 2016). With the planned expansion of mining into the Western Range area (pending approval) there will likely be impacts on the Western Range population.

Defining the plant water-use attributes of the species and its substrate interactions will enable a greater understanding of the niche that this plant occupies. Here we focus on the physical structure, hydrology and chemistry of habitat substrates, surrounding micro-climates, and the physiological response of *A*. *quadrata* to variation in these parameters.

The broad research objectives of this program are to:

- 1. Define the plant water-use attributes and substrate interactions of A. quadrata,
- 2. Examine the physiological responses of *A. quadrata* in relation to the topography, hydrology, and soil chemistry of habitats,
- 3. Characterise the niche that *A. quadrata* occupies and establish meaningful biological correlates between habitat suitability and plant performance.

In the first year a baseline species distribution model was developed that used eight high resolution edaphic factors at approximately 25 m<sup>2</sup> resolution. Of these factors the model determined slope, elevation, soil bulk density and silt content strongly associated with predicted habitat suitability for *Aluta quadrata* Rye & Trudgen, with plants modelled to occur predominantly on elevated, rocky slopes, with shallow sandy soils with low silt and clay contents. Additionally, *A. quadrata* is limited in its distribution to soils with a bulk density of 1.37 - 1.43 g/cm<sup>3</sup>. Collectively, these parameters highlight the specific edaphic niche characteristics of *A. quadrata* and provide further evidence to support this species as a slope species.

We used the SDM to inform the selection of sites for ecophysiological studies, by identifying "highsuitability habitat" and "low-suitability habitat" locations with *A. quadrata* present and absent, and by comparing performance (through ecophysiological assessments) with a morphologically similar sympatric species, *Eremophila latrobei* F.Muell. The studies focused on capturing plant productivity, water-use and health as measured through gas exchange, leaf water potentials and chlorophyll flourescence and found the modelled high suitability site with *A. quadrata* present to be characterised by elevated responses for gas exchange, but not always for plant available water in both species. In addition, critical leaf water potential estimates indicated winter dry periods to be a threshold period for plant functioning, as predawn leaf water potentials rapidly decrease with the transition from winter to pre-summer dry periods. During these periods most plants surveyed were also observed to senesce and abort flower development.

Ongoing ecophysiological monitoring in the second year will thus explore dynamics in plant functioning, water sourcing and niche impacts in *A. quadrata*. Broadly, the second year focused on: 1) continuing seasonal measurements in *Aluta quadrata* plants in sites that vary in modelled suitability; 2) characterizing plant mineral nutrition in high and low suitability sites; and 3) characterizing water sourcing in plants across an elevation gradient.

## Key performance indicators

Year 2 research focused on ongoing measurement of ecophysiological patterns across seasons that will underpin model refinements (KPI 2 and 3), characterizing differences in soil chemical and physical properties associated with the high and low suitability sites (KPI 5), and characterizing plant water sourcing via isotopic studies (KPI 6 and 7).

**Table 1:** Key performance indicators planned for delivery in the second research year by Kings Park Science.

KPI	KPI Description	Period	Status
2	Refined species distribution modelling completed	Year $1 \rightarrow 3$	In progress
3	Ecophysiological studies – ongoing	Year $1 \rightarrow 3$	Yr 1 and 2 completed, Yr 3 in progress
5	Analysis of soil samples at natural sites completed	Year $1 \rightarrow 2$	Completed
6	Isotopic studies of plant water sourcing in natural sites commenced	Year 2	Completed
7	Isotopic studies of plant water sourcing in natural sites completed	Year 2	Completed, interim report to be submitted in 2023
	Completed field work safely	Year $1 \rightarrow 3$	Yr 1 and 2 completed safely.

## **Disclaimer (Terminology)**

In the report, the SDM is based on probability of occurrence, from which we infer "suitability": the site with a high probability of occurrence is referred to as the 'high suitability site', while the site with the low probability of occurrence is referred to as the 'low suitability site'. By conducting ecophysiological assessments, we can then justify site suitability based on performance of plants. *Eremophila latrobei* assessments are indeed based on the *A. quadrata* SDM; As such, the assessment of this species serves the purpose of exploring the possibility of *E. latrobei* as a suitable 'indicator' for the performance of *A. quadrata* and to assess the potential 'suitability' of sites with *A. quadrata* absent.

## KPI 2 - Refined species distribution modelling

#### Testing of additional spatial layers

Refinement of species distribution modelling is currently in progress. As part of the model refinement, additional spatial layers relating to soil chemistry (nitrogen content, phosphorus content, pH, ferric oxide content) as well as climate (atmospheric temperature, plant available water, soil moisture content) are currently assessed and weighed against the initial high resolution edaphic distribution model.

#### Integrating ecophysiological responses into species distribution modelling

Refinement will also seek to integrate and physiological responses with species distribution models. By relating physiological responses such as productivity (measured through photosynthesis), plant wateruse (measured through stomatal conductance and water-use efficiency), and plant health (electron transport rate and maximum quantum yield, Fv/Fm) with predicted suitability rankings. While first year surveys were confined to only measuring a high and low suitability site for *A. quadrata* plants, secondand third-year studies would seek to measure across an additional five sites that vary in their suitability across the Western Ranges. Field surveys are currently in progress, with the results to be reported in the final study report.

#### KPI 3 and 5 study site selection from high resolution species distribution modelling

Selection of the survey sites was informed by a high resolution habitat suitability model (SDM; see Annual Report 1, Lewandrowski et al. 2022) such that two sites were selected to represent "high-suitability habitat" and "low-suitability habitat" with *A. quadrata* present and a further two sites selected as "high-suitability habitat" and "low-suitability habitat" with *A. quadrata* absent, representing unoccupied niche (Table 2).

Specific details of the SDM are available from "KPI1 – Baseline species distribution modelling" in Lewandrowski et al. (2022). In summary, the edaphic factors that were determined to be the best predictors of the probability of occurrence were associated with slope (%), elevation (m), soil bulk density (g/cm<sup>3</sup>) and silt content (%), such the distribution of *A. quadrata* was modelled to occur predominantly on elevated, rocky slopes. High suitability sites were associated with increased slopes of >15%, elevation between 425-445m, soil bulk density of 1.409-1.410 g/ cm<sup>3</sup>, and low silt contents of < 2%, while low suitability sites were associated with slopes < 10%, elevation between >460 m and <420 m, soil bulk density outside of the ranges between 1.409-1.410 g/ cm<sup>3</sup>, and increased silt contents of >2% (see KPI1 and Appendix 3-8, Lewandrowski et al. 2022).

Ecophysiological assessments (KPI 3) and soil physicochemical properties and plant leaf tissue were monitored in all four sites across (KPI 5). The monitoring period for ecophysiological assessments (KPI 3) were conducted over six monitoring periods ( $22^{nd}-29^{th}$  August 2021,  $24^{th}-31^{st}$  October 2021,  $13^{th}-19^{th}$  March 2022,  $19^{th}-23^{rd}$  May 2022,  $4^{th}-8^{th}$  August, and  $13^{th}-18^{th}$  October 2022). Sampling of soil and plant leaf tissue (KPI 5) occurred only in March 2022, to ensure sufficient leaf material was available for sampling in *A. quadrata*.

**Table 2:** Location and site classification of high and low suitability sites that were selected for ecophysiological monitoring of *Aluta quadrata* and *Eremophila latrobei* in the Western Ranges, Pilbara. Average habitat suitability index (HSI) was calculated from the projected MaxEnt Distribution and N represents the number of plants sampled in each location.

Site	Location (Lat, Long)	Classification	Avg. HSI	Ν
<b>S</b> 1	-23.180062, 117.423802	High Suitability Occupied	74.5	20
S2	-23.177255, 117.420814	High Suitability Unoccupied	79.0	10
S3	-23.180829, 117.427142	Low Suitability Occupied	21.4	20
S4	-23.179032, 117.429558	Low Suitability Unoccupied	28.6	10





## <u>KPI 3 – Ongoing ecophysiological studies</u>

#### Section summary:

- Seasonal monitoring was conducted across six seasons between August 2019 and October 2022, in high and low suitability sites, on *A. quadrata* with the overall objective to validate the edaphic niche model, and further quantify the performance of sites with *A. quadrata* absent using an indicator species (*Eremophila latrobei*). The data collected between August 2019
- *Aluta quadrata* plants were more photosynthetically active in the high suitability site. These sites are also characterised by higher plant water status (as indicated by higher stomatal conductance and transpiration rates), and lower plant water stress (as indicated by higher predawn and midday leaf water potentials). These responses could likely explain higher growth rates in plants in the high suitability site.
- *Eremophila latrobei* plants are more physiologically active that *A. quadrata*, and there are differences in ecophysiological function between sites where *A. quadrata* are present and absent.
- For both species, plant performance showed strong seasonal variation: this means that there are clear, distinct periods of high and low plant functioning. The periods of high plant functioning

generally coincide with summer and post summer rainfall events and are characterised by elevated gas exchange (e.g., photosynthesis, stomatal conductance and transpiration), chlorophyll performance (e.g. Fv/Fm ratios) and leaf water potentials (e.g. pre dawn and midday leaf water potentials), while the periods of low plant functioning coincide with extended periods of dry (e.g. winter transitioning into spring, August-October), and are typically characterised by intense water stress (very negative/ low predawn and midday leaf water potentials) causing a decline in gas exchange and chlorophyll performance.

• Monitoring of *A. quadrata* plants should be focused during the transitioning between periods of high and low ecophysiological functioning to quantify changes in plant performance as they transition into periods of increased stress during the pre-summer dry. These periods also coincide with the largest changes in ecophysiological functioning.

#### Ecophysiological assessments quantified in high and low suitability sites

Ecophysiological evaluation of plant responses to environmental conditions and stressors can provide mechanistic insights into plant performance across sites that have different habitat/niche characteristics (Tomlinson et al. 2021, Valliere et al. 2021). We undertook a suite of measurements (see Table 3) that provided an insight into growth and productivity (photosynthetic rate), plant-water use (stomatal conductance and transpiration rate), plant health (chlorophyll fluorescence indicator for maximum quantum yield -  $F_v/F_m$ ; and electron transport rate, ETR), and plant-water stress (pre-dawn and mid-day leaf water potential).

Trait	Implication of comparative measurement
Photosynthetic rate (A)	High photosynthetic rates may indicate favorable site/environmental conditions or that a given species is suitable for the site. Higher photosynthetic rates are also associated with higher growth rates in plants.
Transpiration $(E)$	High rates of transpiration may indicate favorable plant water status.
Stomatal conductance $(g_s)$	A measure of the degree of stomatal opening which can be used as an indicator of plant water status. Increased stomatal conductance leads to increases in photosynthetic and transpiration rates.
Plant health measured via chlorophyll fluorescence: maximum quantum yield $(F_{\nu}/F_m)$	Provides a measure of photosynthetic and chlorophyll performance and can be used to assess levels of plant stress (e.g., photoinhibition due to high light) using a chlorophyll flouoremeter. $F_v/F_m$ ratios change gradually in response to environmental stresses and are less sensitive to immediate stress in contrast to $A$ , $E$ and $g_s$ . Typical values for healthy plants are $0.8 - 0.85$ . For many wild species in the arid zone, healthy plants are more commonly 0.7-0.8. Stress plants are characteristically <0.6, though these responses must be complimented by other physiological and morphological measures. $Fv/Fm$ measurements are typically undertaken following dark adaptation of leaves for at least 10 minutes (or as determined through a dark adaptation curve).
Electron transport rate <i>(ETR)</i>	Is the actual flux of photons driving photosystem II (PSII) and provides an indicator of photosynthetic and chlorophyll performance, except that this measurement is undertaken on light-adapted leaves using a chlorophyll flourometer, unlike measurements undertaken for Fv/Fm. ETR measurements can be indicative for plant stress, and are sensitive to the ambient temperature, drought and variation in light level at the time of measurement. Values of < 50 µmol electrons m <sup>-2</sup> s <sup>-1</sup> can indicate impaired or low photosynthetic efficiency.
Pre-dawn leaf water potential ( $\Psi_{pd}$ )	Provides an indication of soil water availability, where lower $\Psi_{pd}$ indicates increasing water deficit. This measure typically is quantified prior to first light, when plants equilibrate to the soil water status due to stomatal closure. Measured values can typically range between 0 and -10 MPa. Zero reflects freely available water, -1.5MPa is defined as the permanent wilting point, beyond which moderate and severe drought can occur.
Mid-day leaf water potential ( $\Psi_{md}$ )	Useful for assessing drought strategies across species; drought avoiders will maintain a constant $\Psi_{md}$ while drought tolerators will exhibit a drop in $\Psi_{md}$ . Mid-day leaf water potentials are measured at the peak stress time of day and are typically more negative than their pre-dawn counterparts. Measured values can typically range between 0 and -10 MPa. Zero reflects freely available water, -1.5MPa is defined as the permanent wilting point, beyond which moderate and severe drought can occur.

**Table 3:** Selected physiological plant traits and the implications for plant performance presented in this annual report [adapted from Valliere et al. (2021)]

#### Plant size estimates

We measured plant size characteristics (height, width) to understand if plants at different sizes would demonstrate different ecophysiological performance measures. In summary, there was no relationship between plant size characteristics and gas exchange or chlorophyll fluorescence measures. In addition, we detected no significant annual change in height or width, with plants on average between 124-149 cm in height and 74-128 cm in width in *A. quadrata*, and between 115-180 cm in height and 62-142 in width in *E. latrobei*.



**Figure 2:** Annual measures in plant size estimates (height, and width) in high (light blue ) and low (dark blue) suitability sites where *Aluta quadrata* plants are <u>present</u> and <u>absent</u>. All parameters are plotted as box and whisker plots, indicating minimum, 25<sup>th</sup>, median, 75<sup>th</sup> percentiles, maximum, and outliers in the data. Orange coloured point-estimates within the box and whisker plot are means with standard errors of the mean.

#### *Gas exchange measurements*

For each of the species, photosynthetic rate (A), stomatal conductance  $(g_s)$ , and transpiration rate (E) were measured using a LI-6400XT portable photosynthesis system and gas exchange analyser (LI-COR Biosciences, Lincoln, NE, USA) that was equipped with a 6400-40 leaf chamber fluorometer. All measurements were conducted between 08:00-12:00 pm, representing the time where the plant is most photosynthetically active prior to stomatal closure at solar noon. All measurements were quantified under constant light saturated conditions, whereby photosynthetic active radiation was maintained at 1200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. Additionally, internal carbon dioxide concentrations were equilibrated to 400  $\mu$ mol CO<sub>2</sub> mol<sup>-1</sup> and relative humidity was maintained between 50-70%. Thermal conditions were ambient throughout all measurements to reflect seasonal temperature conditions at the time of measurement. All measurements were quantified on up to 10 replicate plants. On each plant, three replicate measurements were quantified on 2-3 individual tufts comprised of mature needle-like leaves that were located on the terminal stem. For each of the measurements, leaf-tufts were allowed to equilibrate to the internal leaf chamber conditions, whereby the stability of gas exchange parameters was monitored in real-time. Following measurement, leaf-tufts that were measured within the chamber were harvested from the plant and returned to the ecophysiology laboratory for leaf area analysis. All measurements were leafarea corrected prior to statistical analysis.

## Leaf water potential

Leaf water potential measurements were conducted in order to determine plant available water (predawn measurements) and plant water status at the time of stomatal closure (mid-day measurements) (Turner 1981). Predawn ( $\Psi_{pd}$ ) sampling occurred prior to first light (between 0300-0400 am), whereby terminal stems that were approximately 10 cm in length were harvested from plants and stored within a sealed foil bag in cool conditions, prior to leaf water potential assessment. Mid-day ( $\Psi_{md}$ ) sampling

occurred approximately between 1045-1100 am during summer and between 1100-1200 pm in winter, representing the conditions of peak stress and approximate solar noon for the region. All measurements were conducted within 15-30 minutes of harvesting, whereby terminal stems were cut at a 45° angle and immediately secured within a Scholander Pressure Chamber (Model 1000, PMS Instruments Co, USA) with the cut stem externally exposed prior to pressurisation (<100 bar). For each species 10 replicate plants were measured, whereby 2-3 measurements were quantified per plant for  $\Psi_{pd}$  measurements, and a single replicate measurement quantified per plant for  $\Psi_{nd}$  measurements.

#### Chlorophyll performance

Prior to  $\Psi_{pd}$  assessment, chlorophyll fluorescence measurements relating to maximum quantum yield  $(F_v/F_m)$  were quantified using a chlorophyll fluorometer (PocketPI, Hansatech Instruments Ltd, UK) on leaf-tufts for each replicate terminal stem, resulting in 2-3 replicate measurements across 10 plants for each species, per site. Dark adaptation was not required for leaf-tufts, as stems were harvested in the dark during the predawn measurement window. Electron transport rate measures were conducted simultaneous to gas exchange measurements using the leaf fluorometer chamber attached to the LI-6400XT (see above, gas exchange measurements). For ERT-measurements specifically, each of the three replicate tufts was measured a single time, equating to three measurements per plant, per plot

#### Statistical analysis - species-, suitability and site-level comparisons

All ecophysiological parameters (A,  $g_s$ , E,  $F_{\nu}/F_m$ , ETR,  $\Psi_{pd}$ ,  $\Psi_{md}$ ) were analysed by fitting generalised linear mixed effects models (GLMMs), using 'glmer'-function from the 'lme4'-package (Bates 2010, Bates et al. 2015) in the statistical environment, R (R Core Team 2021). To understand species-level differences, GLMMs were constructed on sites where A. quadrata plants were present only (e.g. Sites 1 (high suitability) and 3 (low suitability); Figure 1). For each ecophysiological parameter, we fixed species (A. quadrata; and E. latrobei), site suitability (high; and low) and the monitoring period (August, 2021; October, 2021; March 2022; May, 2022; August, 2022; October 2022), with A. quadrata, the high suitability site and August, 2021 determined as the model intercepts. As we also aimed to understand how sites were performing where A. quadrata plants were absent, the data set was subsetted to only assess performance of the co-occuring E. latrobei across sites (e.g., Sites 1-4). In this scenario, GLMMs were fitted to sites assessing the presence and absence of A. quadrata plants (present, vs absence), site suitability (high, and low suitability sites), and the monitoring period (August, 2021; October, 2021; March 2022; May, 2022; August, 2022). For the E. latrobei site-level comparisons, the model intercepts were defined as sites with A. quadrata plants present, high suitability sites and August 2021. For both species level, and site-level analyses, all main effects, as well all possible two-way and three-way interactions were fitted, followed by assessing model the performance via marginal and conditional R<sup>2</sup>-values (Nakagawa and Schielzeth 2013) as well as graphical analysis of residuals and homogeneity of the model variance. Following model fitting, we calculated Wald Chi-square  $(X^2)$ statistics and performed type-II Wald tests for each fixed effect using the 'Anova'-function in the 'car'package (Fox et al. 2007). All summary statistics from GLMSS are provided in Tables S2-4 in Appendix 2.

#### Results

#### Overall model performance

Of the ecophysiological responses assessed in the GLMMs on a species level (Table S2-4), the traits related to plant water stress were demonstrating the highest model strength with marginal as well as conditional  $R^2$ -values >0.8, followed by traits associated with chlorophyll performance (>0.7), and traits associated with gas exchange (>0.38). For ecophysiological responses across sites for *E. latrobei* plants only, plant water stress (>0.85) and gas exchange (>0.24) were demonstrating higher model strength than traits associated with chlorophyll performance (>0.05).

#### Suitability- and species -level differences

There was strong seasonal variation in plant performance in both species over the six monitoring periods between August 2021 and October 2022 (Figures 3-5). These responses were consistent in models that compare species (Table S4), and between sites with A. quadrata plants present and absent for E. latrobei (Table S5). Elevated plant functioning was measured between March and August, 2022, and stress periods in plant functioning measured between August and October 2021 (Figure 3-5) in both species and across both high and low suitability sites. Maximum performance in both species was observed following periods of rainfall (see. Figure S1, March -August 2022). During this period specifically, plant water stress was low, with both  $\Psi_{pd}$  as well as  $\Psi_{md}$  between -1.2 and -2.4 MPa and -1.8 and -3.3 MPa, respectively. The high plant available water resulted in increased gas exchange rates of  $> 5 \,\mu$ mol  $CO_2 \text{ m}^{-2} \text{ s}^{-1}$  for photosynthesis, > 0.075 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> for stomatal conductance and > 1.5 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> for transpiration (Figure 3), as well as increased chlorophyll performance with maximum quantum yield measures for  $F_{\nu}/F_m > 0.65$  and ETR-responses >80 µmol electrons m<sup>-2</sup> s<sup>-1</sup> (Figure 4). By contrast, the pre-summer dry (peak stress) conditions in October, 2021 represented 50-95% in reductions for ecophysiological traits which resulted in gas exchange rates of  $<2 \mu mol CO_2 m^{-2} s^{-1}$  for photosynthesis, <0.025 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> for stomatal conductance and <0.25 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> for transpiration in both species (Figure 3). These low gas exchange rates were further corroborated by reduced chlorophyll performance with maximum quantum yield measures for  $F_{\nu}/F_m < 0.3$  and decreased ETR-responses  $< 50 \,\mu$ mol electrons m<sup>-2</sup> s<sup>-1</sup> (Figure 4). As well, traits associated with plant water stress indicated low plant available water, with  $\Psi_{pd}$  as well as  $\Psi_{md} < -8$  MPa (Figure 5).

In sites were *A.quadrata* plants were present, there were consistent suitability-level differences measured for gas exchange (*A* <sub>Suitability</sub>:  $X^2 = 15.10$ ,  $gs_{Suitability}$ :  $X^2 = 56.74$ ,  $E_{Suitability}$ :  $X^2 = 26.59$ ; all P < 0.001; Table S2) and plant water stress ( $\Psi_{pd}$  <sub>Suitability</sub>:  $X^2 = 6.10$ , P = 0.01;  $\Psi_{md}$  <sub>Suitability</sub>:  $X^2 = 4.72$ , P = 0.029; Table S2), for both species. Species-level differences were characterised by responses of 6-25% in *A.quadrata* and 17-41% in *E. latrobei* for gas exchange ( $A_{\text{Species}}$ :  $X^2 = 30.43$ , P < 0.001; Table S2); chlorophyll performance (Fv/Fm<sub>Species</sub>:  $X^2 = 90.178$ ,  $ETR_{\text{Species}}$ :  $X^2 = 19.39$ , all P < 0.001; Table S2) and pre-dawn leaf water potentials ( $\Psi_{pd}$  <sub>Site</sub>:  $X^2 = 6.53$ , P = 0.011).

#### Site-level comparison for E. latrobei

There was a strong two-way and three interaction between suitability class and sites where *A. quadrata* are present and absent, and strongly explaining site-level variation for *E. latrobei* (see interaction Sc × P and Sc × M × P, Table S3). In sites where *A. quadrata* plants were absent, there were predicted low suitability sites were characterised by elevated gas exchange, but decreased chlorophyll performance in contrast to high suitability sites– this pattern was the opposite in sites where *A. quadrata* plants were present and this variation was reflected across the six monitoring periods (see Figure 3-4). In addition, differences in plant water stress between presence and absence sites suggest plant available water to vary significantly across the landscape ( $\Psi_{pd}$  presence/absence:  $X^2 = 81.61$ , P < 0.001; Figure 5).



**Figure 3:** Seasonal variation in gas exchange [photosynthetic rate (*A*), stomatal conductance ( $g_s$ ), and transpiration rate (*E*)] over six monitoring periods between in August, 2021 and October 2022, representing winter dry, pre-summer dry, and summer wet conditions in high (light blue ) and low (dark blue) suitability sites where *Aluta quadrata* plants are <u>present</u> and <u>absent</u>. All parameters are plotted as box and whisker plots, indicating minimum, 25<sup>th</sup>, median, 75<sup>th</sup> percentiles, maximum, and outliers in the data. Orange coloured point-estimates within the box and whisker plot are means with standard errors of the mean.





**Figure 5:** Seasonal variation plant water stress [predawn leaf water potential ( $\Psi_{pd}$ ), and midday leaf water potential ( $\Psi_{md}$ )] over six monitoring periods between in August, 2021 and October 2022, representing winter dry, pre-summer dry, and summer wet conditions in high (light blue) and low (dark blue) suitability sites where *Aluta quadrata* plants are <u>present</u> and <u>absent</u>. All parameters are plotted as box and whisker plots, indicating minimum, 25<sup>th</sup>, median, 75<sup>th</sup> percentiles, maximum, and outliers in the data. Orange coloured point-estimates within the box and whisker plot are means with standard errors of the mean.

#### **Interpretations of the trends:**

<u>Model performance</u>: The larger increase in the conditional R<sup>2</sup>-values compared to the marginal R<sup>2</sup>-values for traits associated with gas exchange in contrast to the other ecophysiological traits are a consequence of the random error structure in the model, which is based on the measurement regime undertaken during field surveys: replicate measurements were conducted within plants on a leaf-level, and then between plants on a site and monitoring period-level. The measured variation suggests that there is within as well as between plant-level variation across species and monitoring periods – this supported by the increase in R<sup>2</sup>-value between the marginal and conditional model to >0.65. However, in general the model strength across all traits is considered substantial. Of all of the traits measured,  $\Psi_{pd}$  and  $\Psi_{md}$  were providing the strongest and most consistent predictors.

Evidence of defined seasonal trends: Ecophysiological monitoring identified peak (i.e., elevated plant functioning; see March, 2022) and stress periods (i.e., lowest plant functioning; see October, 2021) for plant functioning in species across both high and low suitability sites (Figure 4), though there was elevated performance in the high suitability site. The highest rates of change over the course of the two-year study occurred in the transitioning between the post summer rainfall period (March-May) and presummer dry (e.g., October). Our study coincided with a dry 2021 transitioning between August and October (see more negative predawn and midday leaf water potentials), compared to the same period in 2022. This drier period in 2021 defined by overall reduced productivity (lower photosynthetic rates) and reduced chlorophyll performance (Fv/Fm; and ETR), which qualified high rates of leaf senescence and low flower production in *A. quadrata*. By contrast, the wetter March- October 2022 period (see elevated leaf water potentials and gas exchange rates) was characterised by overall lower leaf senescence and significant flowering of individuals across the range. This suggests flowering to coincide with water availability extending through summer and autumn seasons to ensure increased productivity, as well as reproductive investment of plants.

Despite the differences in photosynthesis between high and low suitability sites, we found no annual changes in plant size measures in *A. quadrata*. This result indicates that plants, rather than investing into growth of terminal stems (though stem growth likely occurring at a very slow growth rate), are more likely investing into small incremental changes in growth by replacing senesced leaves, and/ or flower production. Third year research will focus on measuring seasonal changes in growth rates as to capture growth as well as contraction of plants.

<u>Species differences:</u> *Eremophila latrobei* plants were on average more responsive than *A. quadrata* across the gas exchange measurements, and were showing differences or opposite trends between high and low suitability sites where *A. quadrata* plants were <u>present</u> and <u>absent</u>, indicating species-level, as well as site-level inconsistencies. Higher gas exchange rates indicate *E. latrobei* plants are more productive, which leads to higher growth and developmental rates compared to *A. quadrata*. This could lead to misinterpretations when considering *E. latrobei* as an indicator species for *A. quadrata*.

<u>Implications for monitoring:</u> Maximum quantum yield (Fv/ Fm) measurements are the most frequently used measures for assessing plant performance, though the variation in performance this parameter has captured, has occurred to a lesser extent compared to the other measures. In our study, we inspected a range of fluorescence-based parameters and included ETR (electron transport rate) in our results, as this parameter provided higher variation and greater sensitivity (similar to gas exchange measurements; see Appendix Figures S7-10) to the seasonal variation observed in the monitoring data. While Fv/ Fm provides a measure for plant condition, ETR-measures deliver an additional insight into the current photosynthetic efficiency of individuals, and thus should be considered for future monitoring (in the absence of gas exchange measurements).

<u>Future considerations:</u> The ecophysiological monitoring has provided confidence to the edaphic SDM model reported in Lewandrowski et al. (2022), however greater resolution of ecophysiological functioning across the range may increase the variation observed between high and low suitability sites. As such, studies in the third year will span across a wider range of sites to further test the edaphic SDM.

## <u>KPI 5 – Analysis of soil physicochemical properties and plant tissue samples</u> <u>at natural sites</u>

## Section summary:

- Soils and plants were generally characterised by low in nutrient status across the range, *A. quadrata* likely adapted to grow on nutrient poor soils. It is unknown if the species is sensitive to changes in nutrient status.
- *Aluta quadrata* and *E. latrobei* plants demonstrated different nutrient status with increased nutrient concentrations for nitrate, nitrogen, potassium, zinc, magnesium and chloride in *E. latrobei* plants. While *E. latrobei* may co-occur frequently with *A. quadrata*, and present similar morphological traits, the combination of dissimilar nutrient statuses and physiological performances between the species would suggest that performance of *E. latrobei* cannot be used to accurately infer performance of *A. quadrata*.
- In *A. quadrata*, the high suitability site had soils with slightly higher pH (low acidity, close to neutral) and higher clay contents compared to the low suitability site. High suitability sites also demonstrated higher plant magnesium and calcium percentages, as well as reduced plant and soil phosphorus concentrations.
- Redundancy analysis indicated that the strongest driver of variation in the physiological trait dataset measured during the March 2022 period was plant and soil phosphorus, followed by plant calcium. Higher concentrations of phosphorus in the soil and leaf tissues generally correlated with lower physiological performance while increases in calcium concentrations were correlated with increases in physiological performance. However, controlled studies to understand how *A. quadrata* physiology is impacted by nutrient availability and soil pH would be required to unravel the mechanisms of these observed trends.

## Soil physicochemical properties and plant mineral nutrition in high and low suitability sites

Measures of soil physicochemical properties and plant mineral nutrition provide an indicator for plantsoil nutrient dynamics, and thus critical determinants for deficiencies, as well as toxicities impacting plants in their surrounding landscape (Lambers et al. 2019). Given the nutrient impoverished condition of soils across Western Australia, understanding soil and plant properties may provide an insight into soil fertility shaping plant-soil interactions, and adaptations for growth and survival, particularly in rocky environments (Lambers et al. 2010). BIF landscapes are typically characterised by iron-rich rocks, but overall low nutrient availability, and their soil properties can often differ from those found in the surrounding landscape. In addition, measurements for soil and plant properties may further provide ground-truthing of the edaphic factors measured in the SDM model.

Soil physicochemical and plant leaf nutrient content were sampled in *A. quadrata* and a morphologically similar sympatric species, *Eremophila latrobei* during the period of highest productivity between 13<sup>th</sup>-19<sup>th</sup> March 2022. This period was chosen as plants were not in their senescence stage, and to ensure that sufficient material could be sampled from individual plants.

## Soil chemical and physical analysis

Soil samples were collected at 10-20cm depth for representative plants at each site during March 2022. Soil samples were dried and sieved to two mm particle size whereby larger rock fragments and woody debris > 2 mm were removed from samples. After sieving, chemical properties were determined by CSBP Soil and Plant Analysis Laboratory (Bibra Lake, WA). The edaphic traits measured include: Ammonium nitrogen (NH 4 NO3, mg kg<sup>-1</sup>; Rayment and Lyons method 7C2b), nitrate nitrogen (NO 3-, mg kg<sup>-1</sup>; Rayment and Lyons method 7C2b), total nitrogen (%, modified Rayment and Lyons method 7A5), Colwell phosphorus (P, mg kg<sup>-1</sup>; Rayment and Lyons method 9B), Colwell potassium (K, mg kg<sup>-1</sup>; Rayment and Lyons method 10D1), soil pH in H<sub>2</sub>O (Rayment and Lyons method 4A1 ) and CaCL2 (Rayment and Lyons method 4B4), electrical conductivity (dS/m; Rayment and Lyons method 3A1), trace elements including copper (Cu, mg kg<sup>-1</sup>), zinc (Zn; mg kg<sup>-1</sup>), manganese (Mn; mg kg<sup>-1</sup>), iron (Fe; mg kg<sup>-1</sup>) via DTPA (diethylenetriaminepentaacetic acid) micronutrient extraction (Rayment and Lyons method 12A1), boron (B, mg kg<sup>-1</sup>; Rayment and Lyons method 12C2), organic carbon (%; modified Rayment and Lyons method 6B1), exchangeable aluminium (Al, meq kg<sup>-1</sup>; Rayment and Lyons method 15G1), along with other exchangeable cations including calcium (Ca; meq kg<sup>-1</sup>) magnesium (Mg, meq kg<sup>-1</sup>) and sodium (Na, meq kg<sup>-1</sup>) using a 1:5 soil: water extraction (modified Rayment and Lyons Method 5A4). Calcium: Magnesium ratio, Effective cation exchange capacity (ECEC) and exchangeable sodium percentage (ESP) were also calculated. Soil texture was assessed via an in-house method of physical characterisation of the soil. Clay, silt, sand and gravel percentages were calculated in-house along with soil colour.

## Plant nutrient analysis

Plant tissue samples were collected from representative plants at each site during March 2022. Plant samples were refrigerated until return to Kings Park Science Laboratory where they were oven dried and weighed prior to analysis at CSBP Soil and Plant Analysis Laboratory (Bibra Lake, WA). Samples were not washed prior to preparation but were ground prior to analysis. Plant tissue analysis included results for: Nitrate nitrogen (NO 3-, mg kg<sup>-1</sup>; CSBP in-house extraction) and total nitrogen (N; %; modified Rayment and Lyons method 7A5), along with the plant mineral nutrients, magnesium (Mg, %), phosphorus (P;%), potassium (K; %), sulfur (S; %) and sodium (Na; %), calcium (Ca; %) and chloride (Cl-; %), copper (Cu; mg kg<sup>-1</sup>), iron (Fe; mg kg<sup>-1</sup>), manganese (Mn; mg kg<sup>-1</sup>), boron (B; mg kg<sup>-1</sup>) and Zinc (Zn, mg kg<sup>-1</sup>). Plant mineral nutrients were all read by inductively coupled plasma (ICP) spectroscopy following McQuaker et al. (1979).

## Statistical analysis

All statistical analyses and ordinations were performed in the R statistical environment (version 4.1.3) using RStudio Version 2022.02.0 (R Development Core Team 2022). Summary statistics are presented as means  $\pm$  standard error for species and site level differences.

## Patterns of variation between sites and species

Principal Components Analysis (PCA) was used to identify and visualise the major dimensions of variation in the soil physiochemical dataset and the plant nutrient datasets between sites and species using the *PCA* function in the '*FactoMineR*' package (Lê et al. 2008). The PCA was carried out on log-transformed data and correlations with the first two principal components was assessed using the *dimdesc* function.

A Bray-Curtis dissimilarity matrix was used for a non-parametric permutational multivariate analysis of variance (PERMANOVA; Anderson 2005) to identify significant differences between the multivariate characterisation of edaphic traits and plant nutrient compositions between sites and species. This analysis was conducted using the *adonis* function in the "*Vegan*" statistical package and *Post hoc* pair-wise comparisons between trait assemblages were conducted to identify the multivariate differences between sites using the *pairwise.adonis* function in the '*pairwiseAdonis*' package (Anderson 2014; Martinez 2019).

## Physiochemical drivers of plant physiology

Redundancy analysis (RDA) was used to construct an ordination of the variation in ecophysiological trait composition as constrained by the soil physiochemistry and plant nutrient status using the *rda* function in the *"vegan"* statistical package (Oksanen et al. 2007). Beginning with an intercept-only model, we applied a step-wise selection procedure using the *ordistep* function to determine the most parsimonious subset of explanatory environmental variables for both models. Model choice was based on Akaike's Information Criterion as a measure of model parsimony. Models and individual axes were tested for significance using the *anova.cca* function. Vectors were superimposed onto RDA biplots with

significance established through 999 permutations using the *envfit* function also in the '*vegan*' statistical package (Oksanen et al. 2007).

#### Results

#### Patterns of variation between sites and species

Multivariate analysis demonstrated that soil physiochemistry varied significantly between sites (*Pseudo-F*<sub>3,54</sub> = 2.53, P = 0.012), but not between species (*Pseudo-F*<sub>1,56</sub> = 1.61, P = 0.185). However, plant nutrient status varied significantly between both sites (*Pseudo-F*<sub>3,56</sub> = 5.61, P = 0.001) and species (*Pseudo-F*<sub>1,58</sub> = 244, P < 0.001). *Eremophila latrobei* plants generally demonstrated higher nutrient concentrations for nitrate, nitrogen, potassium, zinc, magnesium and chloride (see Table S10, Appendix 4), indicating the species to have different mineral nutrition. As such, further analysis was restricted to *A. quadrata* only.

The first two components of the PCA describing soil physiochemistry of *A. quadrata* only accounted for 76.3% of variation (Figure 6). The first axis accounted for 57.8% of this variation and presented strongest correlations with electrical conductivity ( $R^2 = 0.98$ , P < 0.001), while the secondary axis accounted for 19.8% and presented strongest correlations with soil pH ( $R^2 = 0.88$ , P < 0.001). Generally, soils in the high suitability site were characterised by having higher pH and clay contents, but reduced nutrient availabilities compared to the low suitability site (Figure 6).



**Figure 6:** Principal component analysis (PCA) biplot showing the multidimensional differences in soil physiochemical properties between high suitability (red) and low suitability (blue) sites in *A. quadrata*. The data are presented as centroids and standard errors. The proportion of variance explained by each component axis appears in parentheses. Vector loadings per soil physiochemical trait correspond to; soil pH (pH), nitrate (NO 3-), phosphorus (P), sodium (Na), potassium (K), sulfur (S), magnesium (Mg), calcium (Ca), copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), aluminium (Al), boron (B), organic carbon (OC), Silt (%), Clay (%), Sand (%), Calcium: Magnesium ratio (Ca:Mg) , Effective cation exchange capacity (ECEC) and exchangeable sodium percentage (ESP). However, some vector labels have been omitted for visualisation purposes due to overlap.

The first two components of the PCA describing plant nutrient status for *A. quadrata* accounted for 76.9% of variation (Figure 7). The first axis accounted for 60.5% of this variation and presented strongest correlations with plant calcium ( $R^2 = 0.99$ , P < 0.001) and total nitrogen ( $R^2 = 0.98$ , P < 0.001) while the secondary axis accounted for 12.4% and presented strongest correlations with plant boron ( $R^2 = 0.82$ , P < 0.001). Plants from the high suitability site generally had reduced mineral nutrients compared to plants from the low suitability site (Figure 7). However, they did present greater percentages of magnesium and calcium (see Table S11, Appendix 4).



**Figure 7:** Principal component analysis (PCA) biplot showing the multidimensional differences in plant mineral nutrition between high suitability (red) and low suitability (blue) sites in *A. quadrata*. The data are presented as centroids and standard errors along with individual plant loadings. The proportion of variance explained by each component axis appears in parentheses. Vector loadings per plant mineral nutrition correspond to phosphorus (P), sodium (Na), potassium (K), sulfur (S), magnesium (Mg), chloride (Cl), calcium (Ca), copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), aluminium (Al), boron (B). Some vector labels have been omitted for visualisation purposes due to overlap.

#### Physiochemical drivers of plant physiology

We used redundancy analysis (RDA) to understand how the multivariate structure of physiological plant traits can be explained by the physiochemical properties of soil and plant nutrient status sampled in a paired design for each representative plant. After the forward selection procedure, seven factors were identified within the combined explanatory data set. These factors included soil properties of pH, nitrate nitrogen content (mg/kg), soil phosphorus content (Colwell method; mg/kg), exchangeable aluminum (meq/100g) and silt content (%), while plant nutritional properties included plant phosphorus (%) and calcium (%). The RDA model constructed constrained 87.9% (R<sup>2</sup> = 0.879, adjusted R<sup>2</sup> = 0.794) of the weighted variance (inertia) in the physiological trait data. Of this, 63.8% and 14.6% of inertia was accounted for by the first two axes (Figure 8, RDA1: *Pseudo-F*<sub>1,10</sub> = 52.7, *P* = 0.001; RDA2: *Pseudo-F*<sub>1,10</sub> = 12.10, *P* = 0.012). Vector analysis indicated that the strongest driver of variation in the physiological trait dataset was plant phosphorus (R<sup>2</sup> = 0.48, *P*= 0.003), followed by soil phosphorus (R<sup>2</sup> = 0.52, *P*= 0.010) and plant calcium (R<sup>2</sup> = 0.40, *P*= 0.018). The length of the vectors represents the strength of the relationship between the explanatory variable and the physiological trait data set, and

the direction of the vectors indicates the direction of the maximum variation in the response dataset related to the explanatory variable. Plant calcium was higher in the site with a high suitability (1.4  $\pm$  0.08 %) compared to the low suitability site (Figure 8: 1.2  $\pm$  0.08 %). Phosphorus was both lower in soils (Figure 8: 9.4  $\pm$  0.96 mg/kg) and plants (Figure 8: 0.04  $\pm$  0.00) in the high suitability site compared to the low suitability site (soils: 37.8  $\pm$  9.6; plants: 0.05  $\pm$  0.00).



**Figure 8:** Redundancy analysis (RDA) biplot showing *Aluta quadrata* physiological trait composition as constrained by plant mineral nutrition and soil nutrient factors as defined by habitats high suitability (red) and low suitability (blue). The proportion of variance explained by each axis appears in parentheses. Points reflect individual plant estimates with centroids paired with standard errors. The edaphic factors used in these analyses were the ones revealed to be the most relevant following a forward selection procedure using the *ordistep* function in the R statistical package '*vegan*'. Vector labels correspond to plant calcium (Ca), soil pH (pH), plant and soil phosphorus (P), soil aluminum (Al), soil nitrate (NO 3-) and silt (%).

#### **Interpretations of the trends:**

<u>Site-level differences in plant nutrition, but not in soil chemistry</u>: Overall, soils sampled across sites were similar to each other, and characterised as nutrient poor (low fertility). The similarity between the high and low suitability sites is likely explained by the similar characteristics of the ecotypes: both were situated adjacent to creek-lines, away from major drainage channels, and at elevated positions in the landscape. Given *A. quadrata* plants are also found within gullies and creek lines, these ecotypes could harbour different nutrient dynamics, particularly following hydrological changes and sedimentation. Further sampling of these ecotypes could provide a critical baseline understanding for nutrient status across the range.

Differences in plant properties were observed, indicating possible differences in plant mineral nutrition. Generally, *E. latrobei* plants are presenting higher nitrate, phosphorus, potassium, sulfur, copper, manganese, zinc, magnesium and chloride concentrations in their leaf tissue samples, while *A. quadrata* were presenting higher sodium, and iron. *Eremophila latrobei* plants have a wider distribution across

the landscape, which is likely explained by the plant displaying higher productivity (higher photosynthesis rates, as demonstrated by seasonal ecophysiology data in KPI 3) and the capacity to allocate higher concentrations of nutrients from soils in their leaves. While *E. latrobei* may co-occur frequently with *A. quadrata* across the Western Ranges, and present similar morphological traits, the combination of dissimilar nutrient statuses and physiological performances between the species would suggest that performance of *E. latrobei* cannot be used to accurately infer performance of *A. quadrat* in sites where *A. quadrata* plants are absent.

The increased sodium and iron concentrations in *A. quadrata* are likely explained by the plants growing out of more rocky substrates, where salt and iron are available at higher concentrations compared to less rocky soil substrates where *E. latrobei* plants are occurring. It is unknown if *A. quadrata* plants are sensitive to changes in soil nutrients. Changes in soil pH through runoff or erosion could lead to different mineral nutrition status, particularly with regards to phosphorus. Understanding impacts of soil nutrients could be critical, particularly if hydrological changes in the landscape are causing increases in runoff and changes to the nutrient status across the range.

<u>Phosphorus and calcium as determinants for physiological performance</u>: Redundancy analysis indicated plant and soil phosphorus, as well as plant calcium to be the strongest drivers in the variation in the physiological trait data set reported for the March 2022 period (see March 2022 responses, Figures 3-6). Higher phosphorus content in the soil and leaves was generally associated with decreased physiological functioning, while increases in leaf calcium was associated with increased physiological functioning. It is possible that there are mycorrhizal associations, given the presence of C4-grasses, such as *Triodia epactia*, in the immediate vicinity of *A. quadrata* plants. This strategy could explain similar plant phosphorus concentrations in *A. quadrata* leaves in high and low suitability sites, despite lower soil phosphorus concentration in the high suitability site. Further research to identify mycorrhizal associations would be necessary to confirm this hypothesis. While both calcium and phosphorus play an important roles for plant ecophysiological functioning, it is also possible that plants situated on substrates in the high suitability sites can take up nutrients more readily due to the more favourable soil pH (~5.98 ± 0.1). This, in addition to the more favourable edaphic factors, such as slope, silt and bulk density from the SDM-model could provide a more favourable niche for plants to persist in.

## KPI 6 and 7 – Isotope analyses: water sourcing in Aluta quadrata

## Sampling for isotopes across sites of varying suitability and over an elevation gradient

Sampling was conducted in three sites spanning between close to the ridge top, representing populations at the high positions in the landscape (Site 3), midslope positions along rocky gullies (Site 5), and creek lines near the bottom of the ridge (Site 6) on the Western Range. The sites also varied in predicted suitability, and were catagorised as low, medium and high suitability, at Site 3, 5 and 6, respectively. Sampling regimes were undertaken after the summer wet period 19<sup>th</sup>-23<sup>rd</sup> of April 2022, and during the pre-summer dry period, 14-18th of October 2022. Approximately, total summer rainfall since the beginning of January had accumulated up to 267.6 mm. The pre-summer dry period was characterised by lower rainfall of 87 mm since the beginning of June and no rainfall in the 4-week window prior to sampling in October. Sampling consisted of harvesting pant foliage, stems as well as soils within a 300 mm radius around each A. quadrata plant. Given the rocky substrate of the surrounding landscape where individuals were sampled, it was not possible to sample soils at consistent depths of > 300 mm from the surface. Therefore, we extracted soil composites samples between 100-300 mm in depth to keep the sampling consistent. From 5 plants at each site, approximately 10 stems of 10 cm in length inclusive of foliage, that equated to approximately 5 g of plant dry-weight. The stems and foliage were separated, followed by rolling and sealing the stems in gladwrap to minimize evaporative or exclude water-loss from the stem matter. All soil and stem samples were placed in 100 ml plastic collection vials that were sealed with a screwcap lid and wrapped tightly with parafilm tape, and then stored in a dark and cool (< 5°C) until isotopic analyses.

All isotopic analyses were undertaken at the West Australian Biogeochemistry Centre at The University of Western Australia (http:/<u>www.wabc.uwa.edu.au</u>). The composition of water samples ( $\delta^2$ H and  $\delta^{18}$ O) was analysed using an Isotopic Liquid Water and Continuous Water Vapour Analyser Picarro 2130i, and the values reported for each isotope in per mil (‰) after normilisation to VSMOW scale (Viena Standard Mean Ocean Water) following a multi-point normilisation based on three laboratory standards, each replicated twice (Skrzypek 2013). Isotopic signatures for  $\delta^{13}$ C were analysed using a continues flow system consisting of a Delta V Plus mass spectrometer connected with a Thermo Flush 1112 via Conflo IV (Thermo-Finnigan/Germany).

Final analyses for  $\delta^2 H$  and  $\delta^{18} O$  are currently being undertaken and will be summarised in an interim report by August 2023.

## Third year studies to be completed

Third year research will focus on completing KPI2 (refined species distribution modelling), KPI 3 (ongoing ecophysiological monitoring) and KPI6/7 (isotopic studies).

The refined SDM will focus on relating ecophysiological responses across seasons with the predicted suitability from the baseline SDM. In addition, we will also report on SDMs that incorporate additional soil chemical properties that have been identified through field sampling (e.g. soil phosphorus, and pH), in addition to modelling climate parameters related to seasonal rainfall and temperature that would underpin ecophysiological functioning across sites.

Ongoing ecophysiological monitoring would be expanded across the range by increasing sampling in two sites to six sites of varying modelled suitability from the SDM, as well as varying landscape positions and ecoptypes. This would increase our understanding of how plants are functioning more broadly across the landscape, as well as increasing the variation in ecophysiological responses for SDM modelling.

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Daily Precipitation – Paraburdoo 2021–2023

**Figure S1:** Daily precipitation at Paraburdoo Mine 2021-2023. Individual bars are daily total precipitation. Data made available from RTIO.

#### Appendix 2: Summary statistics from generalised linear mixed modelling in KPI3

**Table S2:** Summary statistics for ecophysiological responses from GLMMs for contrasts between *Aluta quadrata* (intercept) and *Eremophila latrobei*), high suitability (intercept) and low suitability sites, and the monitoring period (August, 2021(intercept); October, 2021; March, 2022; May, 2022; August, 2022; and October, 2022).

				Gas ex	change			С	hlorophyll	performan	ice	Plant water stress			
			A gs		E		Fv/	Fv/Fm		TR	PD		MD		
Model Terms	df	$X^2$	Р	$X^2$	Р	$X^2$	Р	$X^2$	Р	$X^2$	Р	$X^2$	Р	$X^2$	Р
Species (S)	1	30.43	<0.001	1.16	0.281	3.19	0.074	90.17	<0.001	19.39	<0.001	6.53	0.011	0.57	0.448
Site suitability (Sc)	1	15.10	<0.001	56.74	<0.001	26.59	<0.001	1.59	0.21	2.22	0.137	6.10	0.013	4.72	0.029
Month (M)	5	16.41	<0.01	13.07	0.023	5.77	0.358	30.97	<0.001	2.70	0.746	135.25	<0.001	67.95	<0.001
$S \times Sc$	1	5.94	0.014	0.97	0.324	0.69	0.403	5.70	0.017	1.52	0.217	0.51	0.47	0.32	0.566
S  imes M	5	8.34	0.138	1.69	0.889	17.82	<0.01	133.82	<0.001	24.10	<0.001	29.96	<0.001	7.45	0.188
$Sc \times M$	5	10.28	0.068	47.35	<0.001	29.50	<0.001	24.53	<0.001	15.71	<0.01	200.17	<0.001	30.68	<0.001
$S\times Sc\times M$	5	2.34	0.674	3.77	0.438	4.27	0.510	9.75	0.045	13.40	0.020	17.07	<0.01	11.01	0.051
Observations		327		464		466		590		517		584		245	
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>		0.439/	0.655	0.506/	0.730	0.384/	0.762	0.717/	0.888	0.265/	0.843	0.896/	0.939	0.823	0.901

				Gas ex	change			Cł	ılorophyll p	erformanc	æ	Plant water stress			
		1	4	g	js	]	Е		Fm	ETR		PD		MD	
Model Terms	df	$X^2$	Р	$X^2$	Р	$X^2$	Р	$X^2$	Р	$X^2$	Р	$X^2$	Р	$X^2$	Р
Site suitability (Sc)	1	5.58	0.018	5.27	0.022	10.34	<0.01	1.51	0.218	8.82	<0.01	10.34	<0.001	0.07	0.789
Month (M)	4	188.56	<0.001	485.05	<0.001	344.14	<0.001	8.11	0.087	463.28	<0.001	158.70	<0.001	49.62	<0.001
Presence vs Absence (P)	1	0.52	0.466	2.21	0.137	34.34	<0.001	2.78	0.095	2.95	0.085	81.61	<0.001	3.35	0.067
Sc  imes M	4	3.93	0.415	25.67	<0.001	6.32	0.176	6.64	0.156	22.09	<0.001	186.23	<0.001	22.97	<0.001
$Sc \times P$	1	35.85	<0.001	66.85	<0.001	38.07	<0.001	3.39	0.065	17.42	<0.001	2.23	0.135	1.17	0.278
$M \times P$	4	55.39	<0.001	20.13	<0.001	111.32	<0.001	9.25	0.055	57.14	<0.001	703.84	<0.001	59.77	<0.001
$Sc \times M \times P$	4	34.33	<0.001	42.43	<0.001	71.49	<0.001	13.92	<0.01	20.65	<0.001	93.31	<0.001	13.56	<0.01
Observations		618		692		694		821		757		814		320	
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>		0.423/	0.501	0.573	0.604	0.579	0.629	0.049/	0.065	0.612/	0.672	0.934/	0.971	0.849/	0.936

**Table S3:** Summary statistics for ecophysiological responses from GLMMs for *Eremophila latrobei*) in high suitability (intercept) and low suitability sites, and the monitoring period (August, 2021(intercept); October, 2021; March, 2022; May, 2022; August, 2022; and October, 2022), where *Aluta quadrata* plants are present.



Sites with Aluta plants absent dditional ecophysiological measures captured through monitoring.

**Figure S4:** Seasonal variation chlorophyll concentration (wet chemistry) in leaves over six monitoring periods between in August, 2021 and October 2022, representing winter dry, pre-summer dry, and summer wet conditions in high (light blue) and low (dark blue) suitability sites where *Aluta quadrata* plants are <u>present</u> and <u>absent</u>. All parameters are plotted as box and whisker plots, indicating minimum, 25<sup>th</sup>, median, 75<sup>th</sup> percentiles, maximum, and outliers in the data. Orange coloured point-estimates within the box and whisker plot are means with standard errors of the mean. Data for October 2021 were absent due to null responses from the CCM-300 chlorophyll content meter. This result was largely due to the high plant water stress measured during this period (see Figure 5, KPI 3) that was also characterized by plant senescence.

ts present



**Figure S5:** Scatter plot of the relationship between plant water use (calculated by the difference between midday and predawn leaf water potentials;  $|\Psi$ md-  $\Psi$ pd|) and predawn leaf water potentials. Seasonal measures are indicated by different colours of the point estimates. Critical water potentials at the peak water use threshold ( $\Psi$ crit<sub>peak</sub>) are calculated for each species by fitting a second order-polynomial regression to the data. Measurements during the August 2021 and October 2022 period generally coincided around the  $\Psi$ crit<sub>peak</sub> threshold, with measurements conducted at increasingly more negative values leading to reduced plant water-use for both species.



**Figure S6:** Scatter plot of the relationship between photosynthetic rate and predawn leaf water potentials. Seasonal measures are indicated by different colours of the point estimates from mean responses of individual plants. Photosynthetic rate rapidly decreased in performance at leaf water potentials at < -2.5 MPa in *A. quadrata* and < -4.5 MPa in *E.latrobei* during monitoring periods August and October 2021, and October 2022.



**Figure S7:** Scatter plot of the relationship between stomatal conductance rate and predawn leaf water potentials. Seasonal measures are indicated by different colours of the point estimates from mean responses of individual plants. High stomatal conductance rates are observed at predawn leaf water potentials > -2.5MPa, which generally coincided between March, 2022 and August, 2022. Stomatal conductance rapidly decreased in performance at leaf water potentials < -2.5 MPa in both species (monitoring periods August and October 2021, and October 2022).



**Figure S8:** Scatter plot of the relationship between maximum quantum yield (Fv/Fm ratio) and predawn leaf water potentials. Seasonal measures are indicated by different colours of the point estimates from mean responses of individual plants. The Fv/Fm measurements indicate low plant stress between March, 2022 and August 2022, and in addition during the months of August, 2021 and October, 2022. The Fv/Fm responses show declines in performance at lower predawn leaf water potentials between < -6 MPa in *A. quadrata*, and < -7.5 MPa in *E. latrobei*. These levels of plant water stress are generally high, which suggest both species to be tolerant to water deficit. The low Fv/Fm ratios during the October, 2021 period are further corroborated by the high levels of leaf senescence in plants. Overall, the Fv/Fm measures are not as sensitive to the plant water stress when compared to photosynthesis and stomatal conductance measures. An explanation for this response is due to the Fv/Fm ratios not being instaneaous measurements, and reflect chlorophyll condition in leaves that occurs over time and in response to prolonged environmental conditions.



**Figure S9**: Scatter plot of the relationship between electron transport rate (ETR) and predawn leaf water potentials. Seasonal measures are indicated by different colours of the point estimates from mean responses of individual plants. Electron transport rate declines more closely follow the decline measured in gas exchange. Although this measure is based on chlorophyll performance, the measure demonstrates increased sensitivity to environmental conditions (e.g., plant water stress as measured through predawn leaf water potentials). Given the increased sensitivity, this measure could provide a useful metric for quantifying photosynthetic performance in leaves relative to the current experienced conditions.

#### Appendix 4: Soil physicochemical and plant leaf tissue measures

Table S10: Summary statistics for plant nutrient concentrations for Aluta quadrata and Eremophila latrobei. Sampled from leaf tissue.

Species	Site Su	uitability	Nitrate	Nitrogen	Phosphorus	Potassium	Sodium	Sulfur	Copper	Iron	Manganese	Zinc	Calcium	Magnesium	Boron	Chloride
			mg/kg	%	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	%	%	mg/kg	%
Aluta	High	Site 1	$40 \pm 0$	$1.03\pm0.02$	$0.04\pm0.00$	$0.57\pm0.04$	$0.17\pm0.02$	$0.12\pm0.00$	$2.31\pm0.19$	$436\pm25.0$	$275\pm50.0$	$6.24\pm0.35$	$1.39\pm0.08$	$0.16\pm0.01$	$52.2\pm3.10$	$0.14\pm0.02$
quadrata	Low	Site 3	$40 \pm 0$	$1.07\pm0.02$	$0.05\pm0.00$	$0.56\pm0.03$	$0.14\pm0.01$	$0.13\pm0.01$	$1.97\pm0.19$	$717\pm65.0$	$224\pm19.0$	$6.99\pm0.35$	$1.20\pm0.08$	$0.11\pm0.00$	$53.8\pm3.20$	$0.14\pm0.02$
Г	High	Site 1	$742 \pm 230$	$2.86 \pm 0.21$	$0.09\pm0.01$	$1.93\pm0.22$	$0.01\pm0.00$	$0.24\pm0.01$	$5.07\pm0.36$	$273\pm21.0$	$409\pm60.0$	$38.4\pm8.80$	$1.49\pm0.09$	$0.26\pm0.01$	$59.0\pm5.30$	$1.43\pm0.10$
Eremophila		Site 2	$525\pm99$	$2.96\pm0.09$	$0.09\pm0.00$	$1.85\pm0.12$	$0.01\pm0.00$	$0.29\pm0.01$	$5.50\pm0.30$	$370\pm 20.0$	$264\pm37.0$	$44.5\pm4.40$	$1.59\pm0.07$	$0.27\pm0.02$	$57.0\pm3.10$	$1.55\pm0.13$
lairobei	Low	Site 3	$664 \pm 66$	$3.04\pm0.08$	$0.12\pm0.01$	$1.66\pm0.10$	$0.01\pm0.00$	$0.28\pm0.01$	$5.89\pm0.34$	$341\pm28.0$	$470\pm76.0$	$57.3\pm6.10$	$1.63\pm0.08$	$0.27\pm0.02$	$53.4 \pm 1.90$	$1.59\pm0.09$
		Site 4	$793\pm190$	$2.91\pm0.08$	$0.08\pm0.01$	$1.65\pm0.10$	$0.01\pm0.00$	$0.28\pm0.02$	$5.33\pm0.25$	$494\pm38.0$	$260\pm59.0$	$39.6\pm3.80$	$1.77\pm0.12$	$0.18\pm0.01$	$52.0\pm1.70$	$1.42\pm0.12$

## Table S11: Summary statistics for soil nutrient concentrations sampled at 20cm depth below Aluta quadrata and Eremophila latrobei

Species	Site Sui	tability	Ammonium	Nitrate	Phosphorus	Potassium	Sodium	Sulfur	Copper	Iron	Manganese	Zinc	Calcium	Magnesium	Boron	Aluminium
			mg/kg	mg/kg	mg/kg	meq/100g	meq/100g	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	meq/100g	meq/100g	mg/kg	meq/100g
Aluta	High	Site 1	$7.60\pm0.97$	$4.3\pm1.00$	$9.4\pm0.960$	$0.48\pm0.04$	$0.04\pm0.01$	$6.75\pm0.79$	$1.12\pm0.04$	$29\pm4.00$	$48.4\pm3.80$	$1.98\pm0.31$	$4.34\pm0.47$	$1.11\pm0.07$	$0.56\pm0.04$	$0.09\pm0.03$
quadrata	Low	Site 3	$22.3\pm10.0$	$14.1\pm7.5$	$37.8\pm9.60$	$0.52\pm0.07$	$0.07\pm0.02$	$12.4\pm4.40$	$1.40\pm0.08$	$77.3\pm18.0$	$47.1\pm6.70$	$2.89\pm0.45$	$5.58 \pm 1.40$	$1.11\pm0.17$	$0.88\pm0.22$	$0.29\pm0.09$
Framonhila	High	Site 1	$6.67\pm0.84$	$6.00\pm1.90$	$10.3\pm0.56$	$0.44\pm0.04$	$0.03\pm0.00$	$7.40 \pm 1.10$	$1.24\pm0.10$	$37\pm5.60$	$44.1\pm4.20$	$2.02\pm0.26$	$3.01 \pm 0.74$	$0.95\pm0.11$	$0.48\pm0.09$	$0.30\pm0.05$
latrobai		Site 2	$14.4\pm2.80$	$15.0\pm5.00$	$11.3\pm2.10$	$0.57\pm0.06$	$0.03\pm0.01$	$9.65\pm0.64$	$1.49\pm0.10$	$44.2\pm8.00$	$48.0\pm3.10$	$2.60\pm0.37$	$5.27 \pm 1.50$	$1.04\pm0.05$	$0.59\pm0.04$	$0.26\pm0.06$
iuirobei	Low	Site 3	$7.25\pm0.86$	$6.00\pm0.86$	$15.5 \pm 2.40$	$0.54\pm0.06$	$0.02\pm0.00$	$7.04\pm0.69$	$1.22\pm0.04$	$41.7\pm6.50$	$39.5 \pm 2.80$	$3.09\pm0.48$	$3.77 \pm 0.58$	$0.89\pm0.08$	$0.57\pm0.06$	$0.23\pm0.05$
		Site 4	$10.8\pm1.50$	$9.00\pm1.50$	$7.89 \pm 1.70$	$0.6\pm0.05$	$0.02\pm0.00$	$8.23\pm0.75$	$1.36\pm0.09$	$30.9\pm3.40$	$53.3\pm6.10$	$2.46\pm0.41$	$5.21\pm0.74$	$0.92\pm0.07$	$0.65\pm0.04$	$0.10\pm0.02$

## Table S12: Summary statistics for soil physical properties sampled at 20cm depth below Aluta quadrata and Eremophila latrobei

Species	Site	Suitability	ECEC Meq/100g	Organic Carbon %	<b>Conductivity</b> dS/m	<b>рН</b> (Н2)	Clay %	Silt %	Sand %
Aluta quadrata	High	Site 1	$6.06\pm0.47$	$2.21\pm0.29$	$0.05\pm0.01$	$5.98\pm0.1$	$23.1\pm0.6$	$16 \pm 0.5$	$61 \pm 0.9$
<u>^</u>	Low	Site 3	$7.58 \pm 1.6$	$2.65 \pm 0.33$	$0.1 \pm 0.04$	$5.56 \pm 0.13$	$21 \pm 1.5$	$15.6 \pm 0.66$	$63.4 \pm 1.5$
	High	Site 1	$4.7\pm0.81$	$1.93 \pm 0.44$	$0.05\pm0.01$	$5.48\pm0.05$	$20.6\pm0.72$	$14.5\pm0.19$	$64.9 \pm 0.85$
Eremophila latrobei		Site 2	$7.17 \pm 1.5$	$2.4 \pm 0.26$	$0.08\pm0.02$	$5.64 \pm 0.11$	$24.1\pm0.8$	$15.2\pm0.58$	$60.7 \pm 0.69$
	Low	Site 3	$5.46\pm0.64$	$2.03 \pm 0.22$	$0.05 \pm 0.01$	$5.63 \pm 0.14$	$21.1 \pm 0.82$	$14.2 \pm 0.59$	$64.7 \pm 1.2$
		Site 4	$6.87 \pm 0.81$	$2.5 \pm 0.28$	$0.07 \pm 0.01$	$5.83 \pm 0.06$	$23.5 \pm 1$	$13.7 \pm 0.77$	$62.8 \pm 1.7$