

Supporting information to:

Poorter H, Pons TL & Reichgelt T. 2025. Stomatal density and index are more responsive to light intensity than to [CO₂]; a meta-analysis and implications for paleo-CO₂ reconstruction. *Plant Ecophysiology* **1**: xxxx
Doi: xxx

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Supporting Information S1: Extended Materials & Methods.

Based on the text in the Appendix of Poorter et al. (2022a)

Note that we adhered as closely as feasible to the procedures used by Poorter *et al.* (2019) and Poorter *et al.* (2022b) in analyzing plant responses to light and [CO₂].

A. Experiments considered.

We collected data from published experiments in physiology, horticulture, agronomy, forestry and ecology where plants were exposed to varying CO₂ concentrations, light levels, temperatures or water availability during a substantial part of their experimental life. We considered experiments with autotrophic plant species that have leaves, stems and roots, considering the caudex of rosette plants as well as the leaf sheaths of grasses as analogous to stems. Different experiments followed varied protocols for pre-growing plants, depending on the species' growth rate and seed mass. In some cases, seeds were planted, and pots were directly placed under different levels of the environmental factor of interest. In other cases, plants were first grown under standard conditions and the experiment began 1–4 weeks later. For slower-growing tree seedlings, plants may have been pre-grown for one or two seasons before being placed under different environmental conditions. There is also considerable variation in the duration of experiments, ranging from less than two weeks up to over a year. We focused on experiments where plants had sufficient time to acclimate to the environmental factor under study. As a rule of thumb, we included experiments where treatments lasted for more

than two weeks, and plants developed over 80% of their biomass under those treatments. However, it is important to note that not all experimental reports provided all the necessary information.

Experiments in which plants were only exposed to different levels of the environmental factor of interest in the generative phase were not considered. We included experiments conducted in growth chambers, glasshouses, open top chambers (OTC's) and Free-Air Carbon dioxide Enrichment facilities (FACE). We also considered experiments in the field carried out on monocultures. Following the classification of Körner (2006), we excluded measurements of plants in natural ecosystems. Studies with interspecific competition were also excluded. In cases of factorial combinations with other environmental factors (e.g. ozone, UV-B, water, salinity), we selected the combination of conditions in which control plants performed best. Data from plants in the vegetative, flowering and fruiting stage were included; however, most traits were measured during the plant's vegetative phase.

B. Measurements considered

We considered data for stomatal density (SD) and stomatal index (SI). To stay consistent with the plant's physiology, we summed the SD's reported for the abaxial and adaxial sides of the leaf, and averaged the SI's for both sides. We did not analyze responses of the adaxial and abaxial sides separately. However, when data were available for only one side of the leaf, we used those values.

C. Collecting the data

Data were sourced from tables, graphs (digitized with Engauge digitizer v.

12.1), supplemental material and additional data provided by the authors. As mentioned above, all data collected were averages from one or preferably more harvests, aimed at excluding most plant-to-plant and day-to-day variability. No attempt was made to weight data points based on the number of plants observed or the variability around the mean. This was partly because such information was not always available or clear in the data sources. We chose to adopt a more inclusive approach, as it allows us to draw broader conclusions based on more independent observations. A second consideration is that variability tends to be greater in certain species, such as wild (tree) species, compared to others, like genetically uniform crop species (see Poorter & Garnier, 1996). We did not want the data from wild species to have less weight in the analysis. Thirdly, we also included data from experiments where only two growth chambers or glasshouses were used and CO₂ treatments as well as plants were regularly switched. While these experiments are often criticized for pseudo-replication, due to potential unaccounted differences between chambers (Filion *et al.*, 2000), we reasoned that these additional differences would introduce random variation in CO₂ responses, and would be averaged out in a meta-analysis across multiple experiments. To integrate the extra information from experiments carried out with different genotypes or cultivars, or in somewhat different experiments within a single publication, but without giving those genotypes/experiments too much weight, we limited the inclusion to a maximum of three genotypes and/or experiments per publication. Similarly, while we included data from experiments with different species, we restricted the number to no more than 10 species per experiment. When more than 10 species were reported, we selected the species that were most phylogenetically diverse.

D. Calculations and Statistics

Scaling across experiments. Because there is large species-to-species variation with regard to the absolute phenotypic values, we focused on analyzing relative responses. The procedure was largely as outlined in Poorter *et al.* (2010). Since different experiments subjected plants to varying levels of the environmental factors of interest, we identified the level which is often included in published experiments. This level, which we used as our reference level for the subsequent normalizations, was 450 ppm for CO₂ experiments, 8 mol m⁻² d⁻¹ for the Daily Light Integral (DLI), 20 °C for the 24h averaged temperature, and the water availability where plants were growing best in drought stress experiments. In principle, the exact value of the reference level does not affect the analysis, as long as it falls within the range of most experiments. For each species/experiment combination ('case'), we interpolated to estimate what the value of the phenotypic trait would have been at the reference level, based on data from the two adjacent levels of the environmental factor.

In less than 1% of the experiments (with the exact number depending on the trait under consideration), the reference level was not included within the range of the experiment. These experiments, often including extreme treatments, are very relevant for establishing the dose-response curve (DRC) across the full range of the environmental factor. To include these data, we first fitted a Loess curve (local polynomial regression) through the 99% of the data that could simply be scaled versus the environmental factor of interest. For the experiments that did not include the reference level, we used the phenotypic trait value of the treatment closest to the reference value. We then determined from the Loess curve what the estimated across-species value of

the response ratio was at that specific level. We assumed that this value was also the response ratio for the phenotypic trait at the level closest to the reference level for the species/experiment combination under consideration. We subsequently scaled all other treatments of the same species/experiment combination relative to this 'anchor point'. Finally, we removed the anchor point that was used to anchor the full species/experiment combination to the fitted Loess curve, as it no longer provided independent information after the anchoring process.

Data curation. Errors in the database could result from various sources, including mistakes by authors during the experiments, errors in data evaluation, errors during the writing of the manuscript, and misstep during our digitization process, or missteps during our calculations. Systematic errors that are a consequence of erroneously reporting wrong units (e.g. mm rather than m) do not influence scaled values, as all values were analyzed proportionally, normalized to 1 at the reference level of the environmental factor. However, systematic errors such as other mistakes in the calculations, or mislabeling of variables (e.g. calculating Leaf Mass per Area, but misidentifying it as Specific Leaf Area, which actually is the inverse) poses a more significant issue. In case of doubt, we contacted the original authors for clarification, and changed the data when the authors indicated that something had gone wrong. For all cases, we calculated the overall slope of the scaled phenotypic traits against the environmental factor of interest. Data from cases showing the most extreme responses, i.e. those with the most positive and most negative slopes, were rechecked manually. Subsequently, the 1% of species/experiment combinations with the highest and the 1% with the most negative slope were excluded from the analysis to ensure robustness. As a result, the dose-

response curves presented here are based on 98% of the total data initially collected, reflecting the most reliable and consistent subset of information.

Dose-response curves. Avoiding a-priori assumptions about data distribution and form of the relationship, we first summarized overall relationships as well as normal ranges by binning all the scaled points in 10 groups, based on deciles with respect to the levels of the environmental factor of interest. If fewer than 10 observations were available in each bin, we used proportionally fewer bins. For each bin, we then calculated the median environmental level for all data points, as well as the median and the 10th, 25th, 75th and 90th percentile for the scaled phenotypic trait values. The dose-response curves with the median values per bin visually provide information on the amount of data on which they are based (by the number of bins used) as well as the information density along the dose-response curve (by means of the distance in the x-axis direction among the medians). For further quantitative use, smoothed dose-response curves were derived using all individual data points, which were grown and measured between 0-2000 ppm, 0 – 60 mol m⁻² day⁻¹, 5 – 35 °C, or the scaled biomass from 0.1 – 1 for water -stressed and control plants. We did so by means of quantile regression (package "Quantreg"; Koenker *et al.* 2021), focusing on median values, so as to minimize the effect of outlying observations and avoid assumptions about the distribution of the data. We tested four potential models to describe the relationship between the phenotypic trait Y and the environmental variable X. First a null model of no relationship between Y, and X:

$$Y = a \tag{1}$$

where a is the overall mean value of the trait.

Second a linear one:

$$Y = a + b.X \quad (2)$$

A simple model describing a straight-line relationship where b represents the rate of change in Y for a unit increase in X .

Third, a quadratic relationship:

$$Y = a + bX + cX^2 \quad (3)$$

For many traits the relationship saturated at higher levels. Therefore, we finally used a three-parameter monomolecular equation as applied in Poorter *et al.* (2019):

$$Y = a(1 - b \cdot e^{-cX}) \quad (4)$$

This model describes a saturating response, as often observed for biological traits under increasing environmental stressors. It includes three parameters: a : the asymptotic maximum value of the trait; b : a scaling factor determining the extent of response; c : the rate constant describing the speed of saturation.

To identify the best-fitting model, the Akaike Information Criterion (AIC) was calculated for each equation. The corrected version, AICc, was used to account for smaller sample sizes. Model selection was performed using the R package MuMIn, which ranks models based on their AICc values, with lower values indicating better fit (Bartón, 2024).

In no case did the decile plots show a relationship with a local minimum or maximum.

Plasticity Index (PI). The plasticity index provides a standardized measure of the phenotypic response of a trait across a specified section of the dose-response curve. Following the outline of Poorter et al. (2010), the PI was calculated as the ratio of the maximum to minimum values of the phenotypic trait within defined ranges: 200-1200 ppm in case of CO₂, 1 – 50 mol m⁻² d⁻¹ for light, 5-35 °C for temperature, and 0.1 – 1 for relative size in case of water supply. The ranges were chosen to be as broad as possible, while avoiding strong extrapolations, particularly for traits with limited data coverage. For traits exhibiting negative slopes, the PI was multiplied by -1. The PI thus reflects the fold-change between the highest and lowest value of the fitted dose-response curve, using a unified scale for positive and negative trends. The advantage of the expression of plasticity as a ratio is that for variables that are multiplicatively related, plasticity indices can be multiplied as well. Note that this is not a plasticity index in the traditional sense where the response of a given genotype or species is characterized. Rather, this indicates the phenotypic changes observed across a wide range of plant species, under the assumption that all types of species have been equally well-measured over the range of environmental levels considered.

Consistency Index (CI). To describe how consistent the responses were, we considered in what percentage of the cases (case = one species or genotype measured at various levels of an environmental factor in a specific experiment as described in a specific publication) the plants had a higher value

for that trait at the highest level applied compared to the lowest level. For those cases where the trait values for a specific case were reported identical at the highest and lowest level, we added half of that number to the positive cases, and the other half to the negative cases. CI values close to 50% indicate inconsistent responses, where the trait shows no clear directional changes across cases, or strong differences in direction across different species. In contrast, values near 0% or 100% represent highly consistent responses, with traits consistently decreasing or increasing, respectively, as the environmental factor increases. Note that some traits can have a low PI, but still change in a highly consistent manner.

Reliability Index (RI). The Reliability Index (RI) assesses the confidence in the dose-response curves by integrating four key factors. First, the range of experimental levels contributes significantly, with broader ranges (e.g., 100–2000 ppm for CO₂) resulting in higher reliability compared to narrower ranges (e.g., 350–700 ppm). Second, the number of observations strengthens the reliability, as more data points enhance the robustness of the curve. Third, the number of species studied increases the generalizability of the response; for instance, observations across 300 species are more robust than those based on only two species. Fourth, the variability of the data around the fitted curve is critical; lower variability improves the confidence in the derived DRC. Each of these factors was scaled on a 0–9 scale, and their average was used to calculate the RI, providing a standardized measure of confidence in the presented DRCs. Higher RI values indicate greater reliability.

All data were analyzed with version 4.1.3 of R (R Core Team, 2022).

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Supplemental information 2: References used for the meta-analysis, listed per environmental factor.

References are listed for each of the environmental factors considered separately. Full details on the references can be found in the accompanying paper.

A. [CO₂] (122 references)

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B. Light Intensity (83 references)

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D. Water Availability (33 references)

(Papers where the size of the plants (fresh mass, dry mass, leaf area) after the drought treatment were not reported were not included in the analysis).

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**Supporting information S3:
Detailed figures of the responses of stomatal density and index to [CO₂], Daily Light Integral, Temperature and Water availability.**

Figures show the scaled responses for herbaceous (blue dots) and woody (red dots) separately, and for all data the interquartile range (shaded area), the 10th and 90th percentile (dotted lines), the median value for each 10% of the subsequent data (black line) and the overall fitted relationship (brown line) over the range considered for the Plasticity Index. The dotted line shows for comparison a Loess curve fitted over all datapoints. Other information provided in the graphs is explained in Fig. S0.

Fig. S0 : Explanation of summary plots

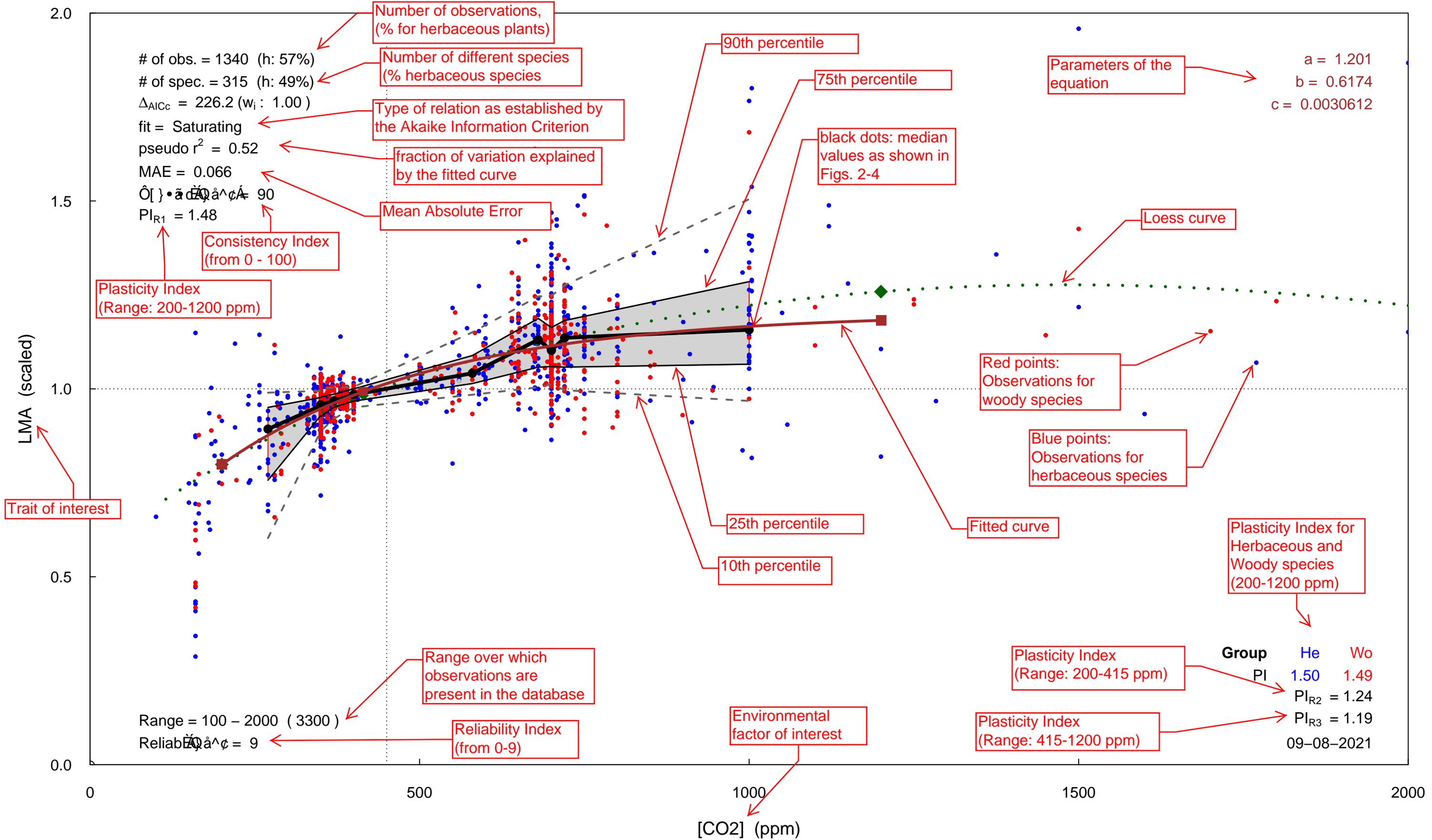


Fig. S01 : Summary plot of Stomatal Density vs. [CO2]

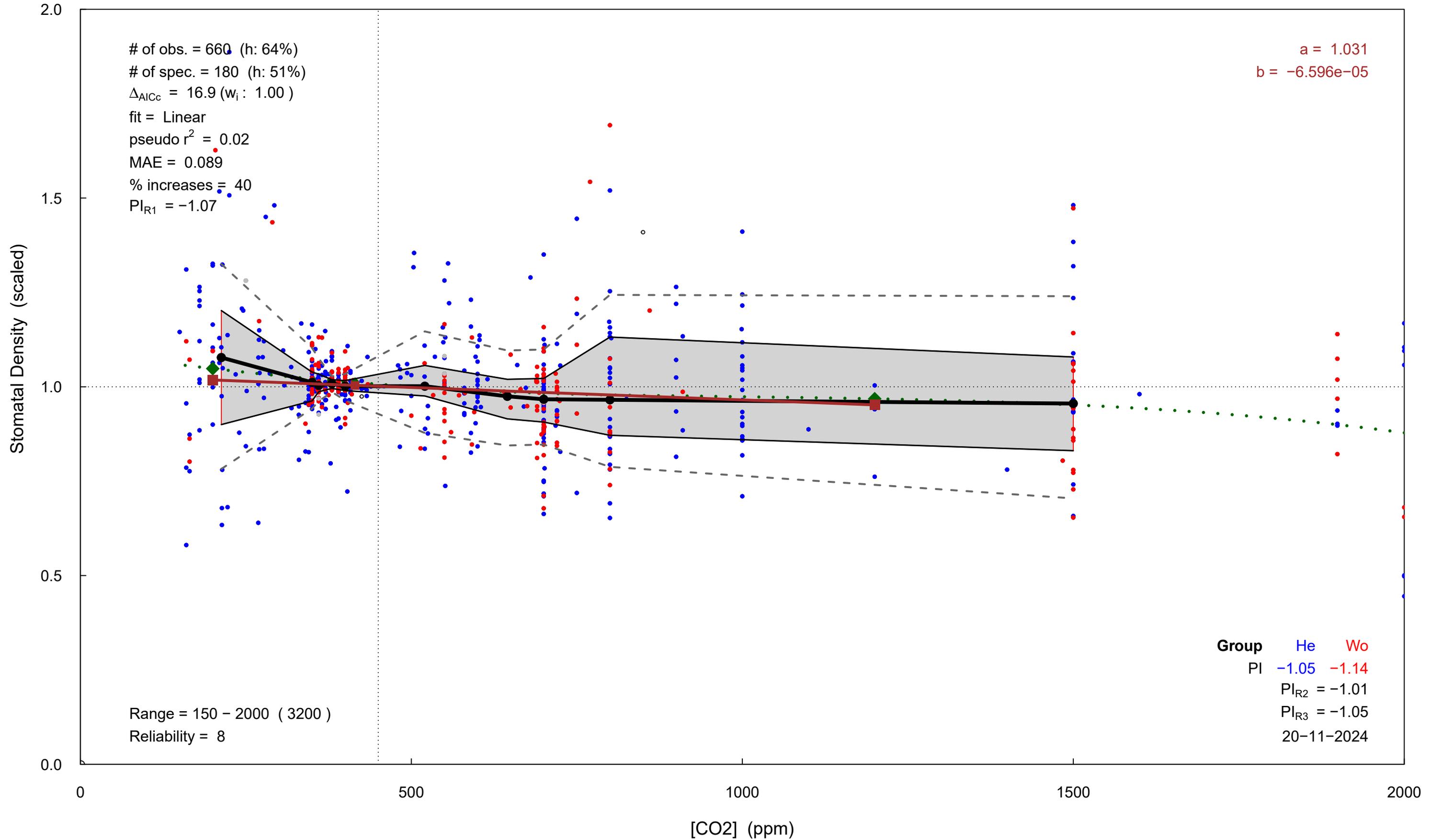


Fig. S02 : Summary plot of Stomatal Index vs. [CO2]

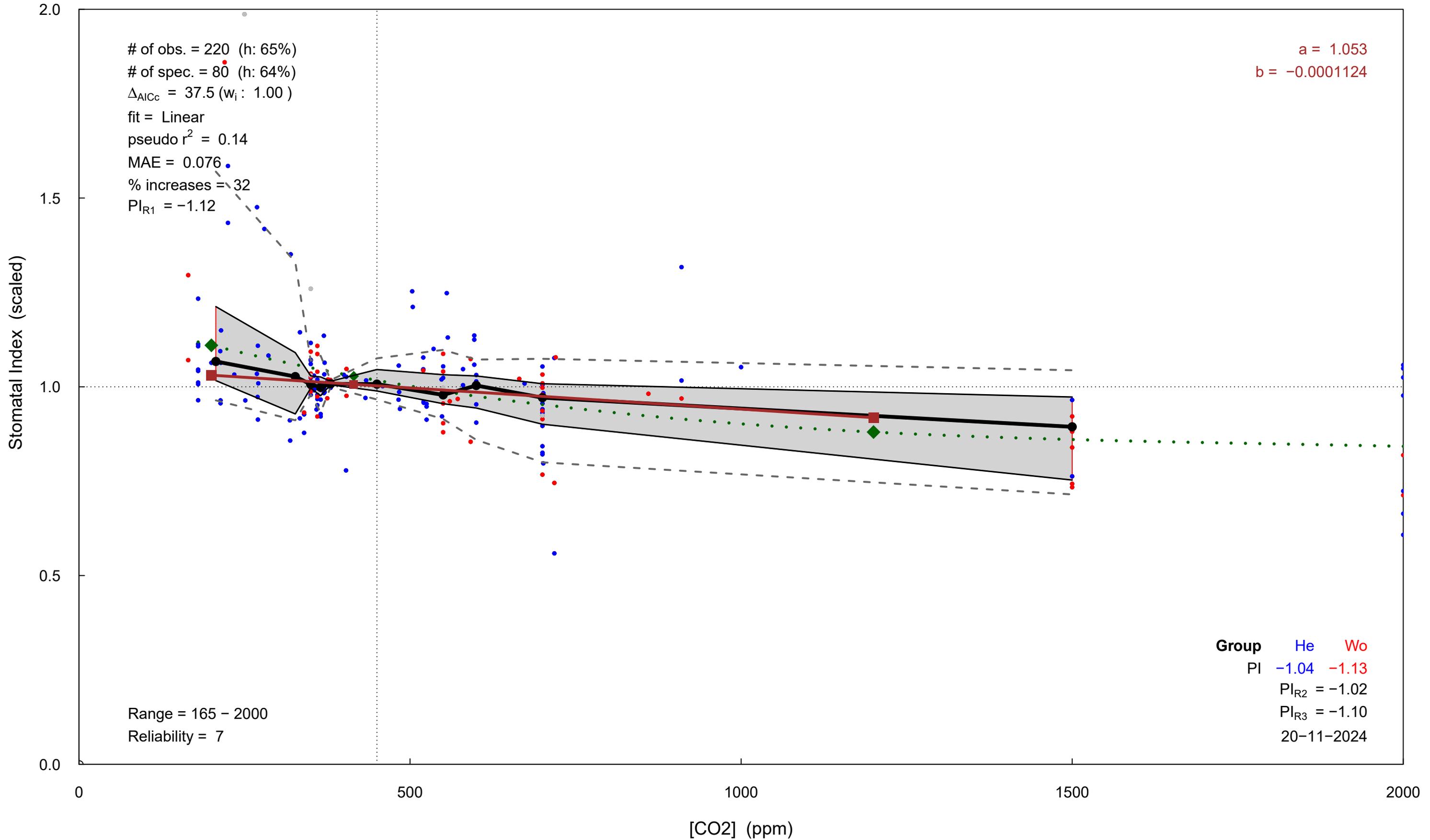


Fig. S03 : Summary plot of Stomatal Density vs. Daily Light Integral

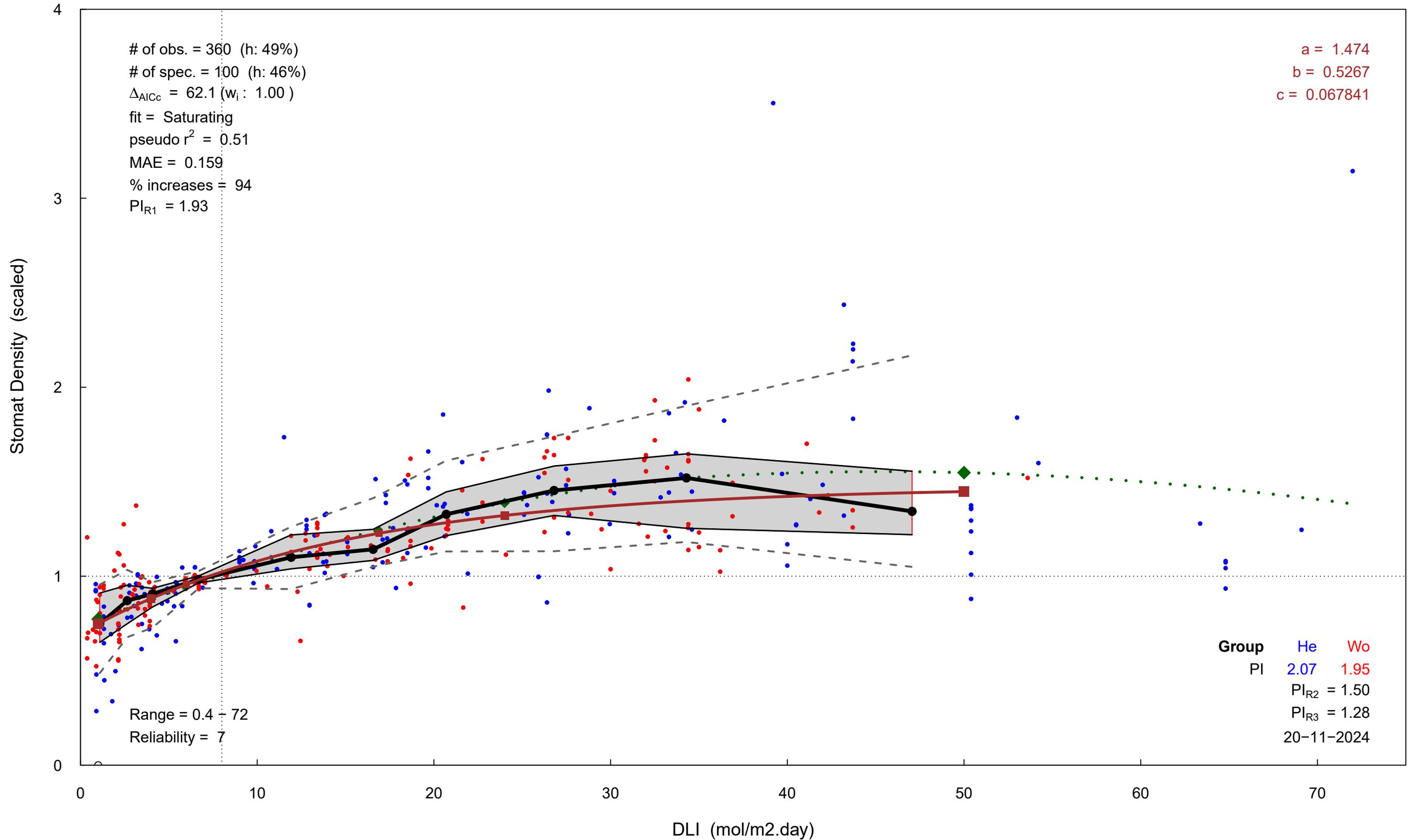


Fig. S04 : Summary plot of Stomatal Index vs. Daily Light Integral

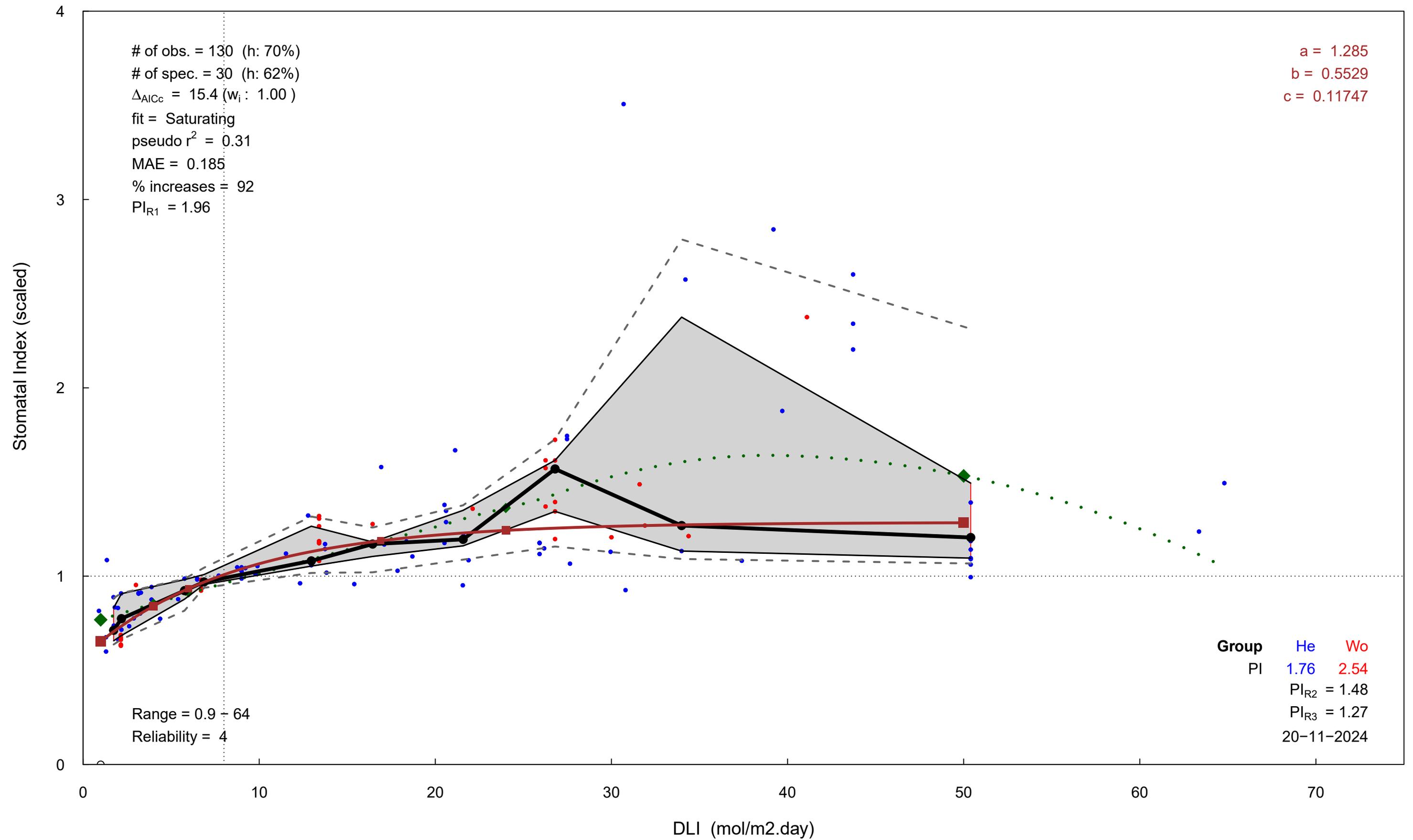


Fig. S05 : Summary plot of Stomatal Density vs. Temperature

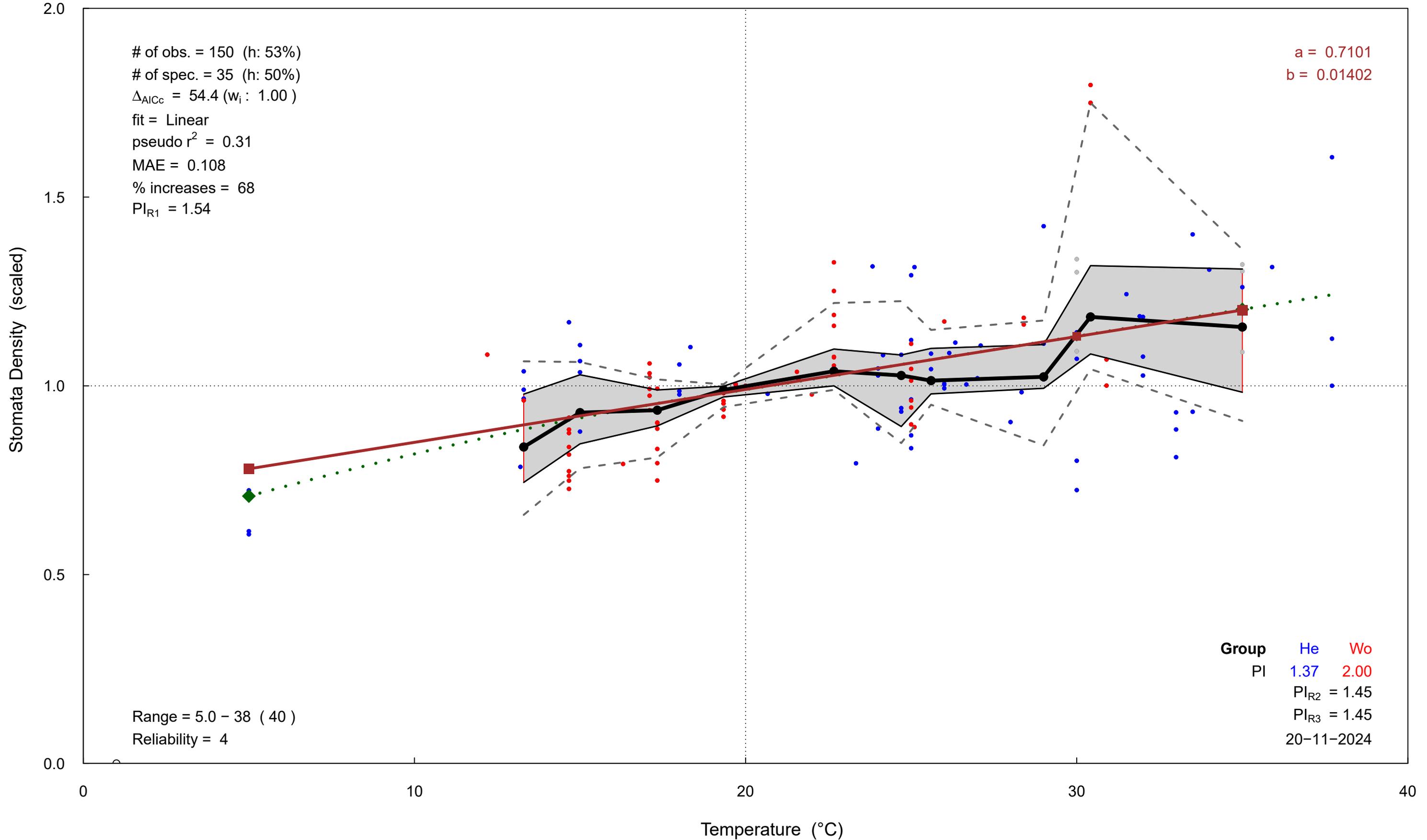


Fig. S06 : Summary plot of Stomatal Index vs. Temperature

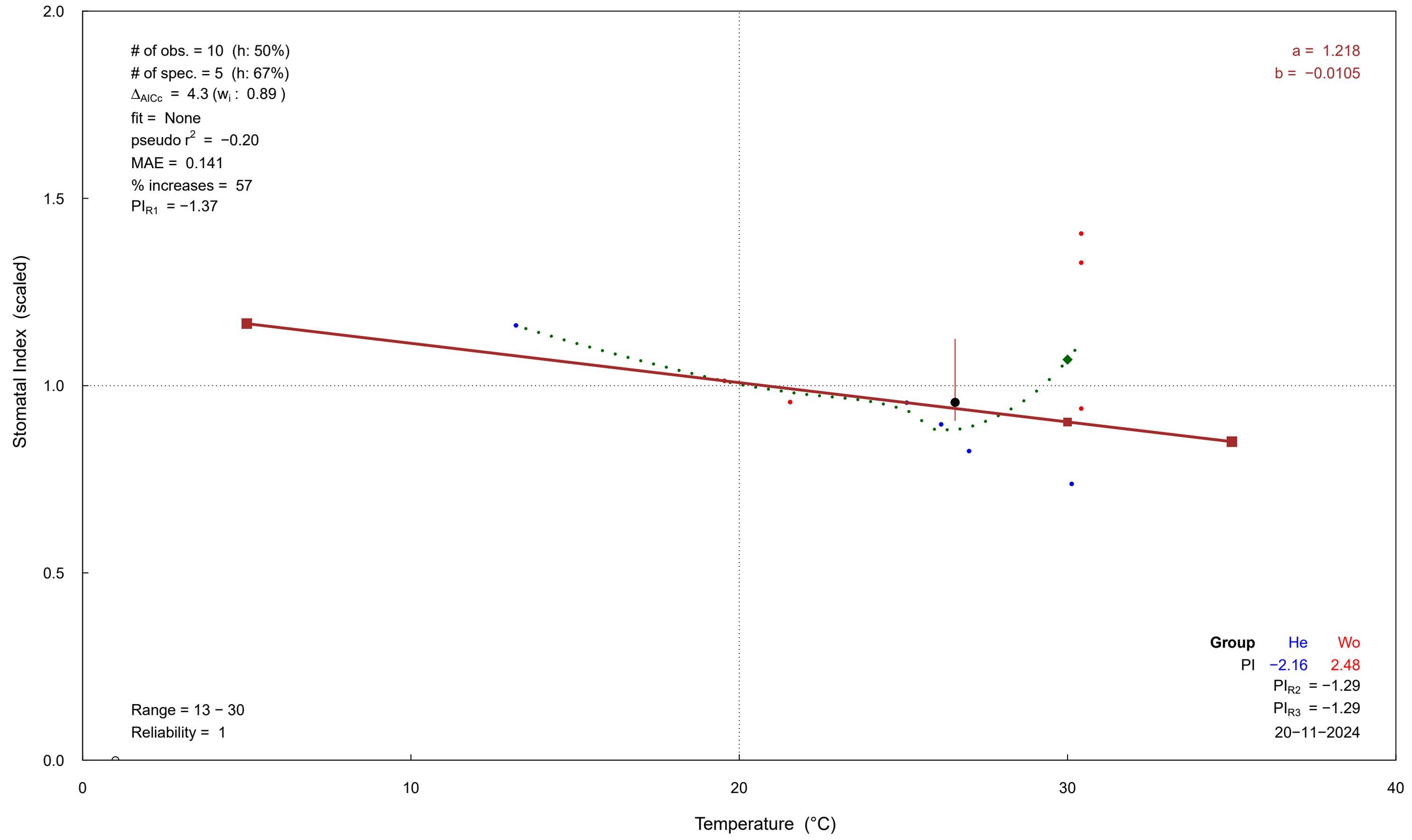


Fig. S07 : Summary plot of Stomatal Density vs. Water Availability

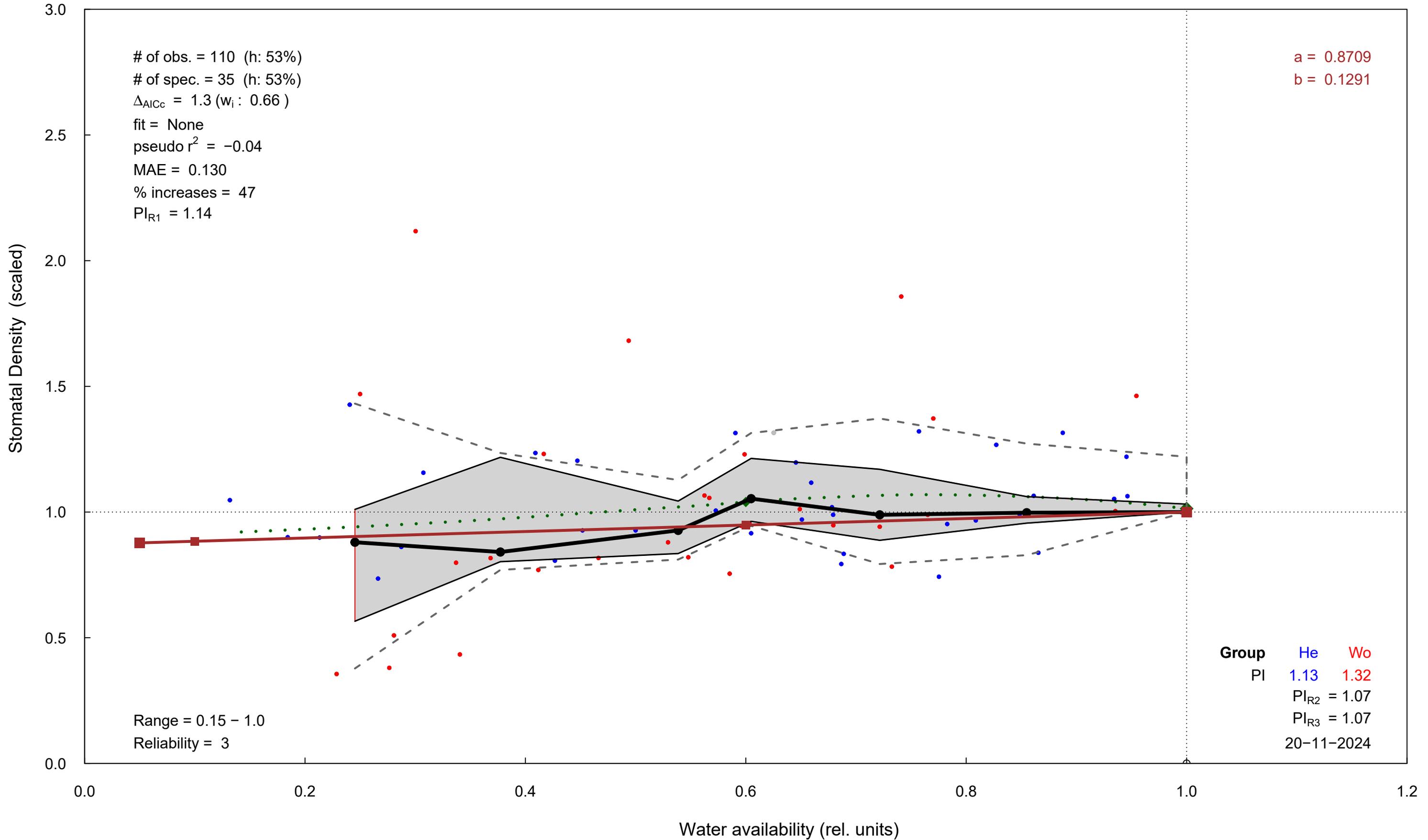
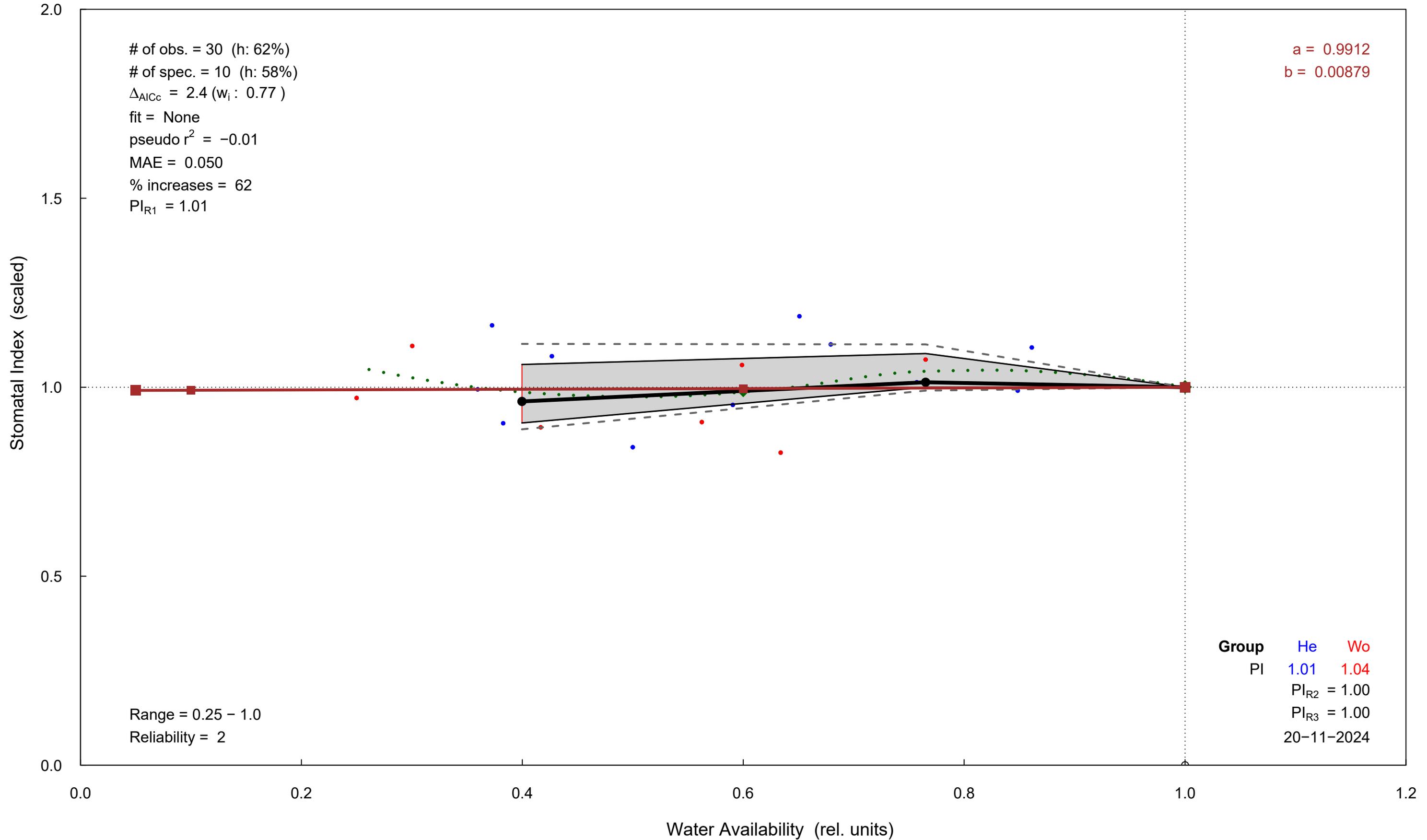


Fig. S08 : Summary plot of Stomatal Index vs. Water Availability



**Supporting information S4:
Responses of various functional groups of species.**

Plasticity Indices (PI) per functional group, along with the number of observations (n), the form of the Dose-response curve (DRC form), and the parameters of the fit (a, b and c). Data are given for both Stomatal Density and Stomatal Index. PI differences between groups have not been tested for significance, unless mentioned in the text.

CO ₂	Functional groups	Stomatal Density						Stomatal Index					
		PI	n	Regform	a	b	c	PI	n	Regform	a	b	c
	All species	-1.07	654	lin	1.031	-6.596E-05	-	-1.12	223	lin	1.053	-1.124E-04	-
	Herbaceous	-1.05	418	lin	1.022	-4.525E-05	-	-1.04	145	lin	1.021	-3.704E-05	-
	Woody	-1.14	225	lin	1.056	-1.231E-04	-	-1.13	69	lin	1.052	-1.159E-04	-
	Herb-C3-Annuals	-1.03	199	lin	1.014	-3.214E-05	-	1.01	50	lin	0.997	1.388E-05	-
	Herb-C3-Perennials	-1.07	118	lin	1.030	-6.761E-05	-	-1.26	52	lin	1.092	-2.140E-04	-
	Herb-C3	-1.06	324	lin	1.028	-6.187E-05	-	-1.04	106	lin	1.021	-3.704E-05	-
	Herb-C4	-1.01	94	lin	1.015	-1.038E-05	-	-1.01	39	lin	1.013	-1.170E-05	-
	Herb-C3-Monocots	-1.03	135	lin	1.017	-3.369E-05	-	1.03	34	lin	0.991	2.857E-05	-
	Herb-C3-Dicots	-1.02	174	lin	1.008	-1.686E-05	-	-1.02	64	lin	1.011	-1.708E-05	-
	Herb-C3-noN2Fixing	-1.05	277	lin	1.020	-4.371E-05	-	-1.04	88	lin	1.022	-3.811E-05	-
	Herb-C3-N2Fixing	-1.08	47	lin	1.036	-7.930E-05	-	-1.21	18	lin	1.080	-1.782E-04	-
	Woody	-1.13	223	lin	1.055	-1.226E-04	-	-1.13	69	lin	1.052	-1.159E-04	-
	Woody-Deciduous	-1.06	94	lin	1.031	-5.719E-05	-	-1.14	35	lin	1.058	-1.238E-04	-
	Woody-Evergreen	-1.14	102	lin	1.055	-1.230E-04	-	-1.12	30	lin	1.051	-1.130E-04	-
	Woody-Angiosperms	-1.16	151	lin	1.066	-1.422E-04	-	-1.27	47	lin	1.105	-2.271E-04	-
	Woody-Gymnosperms	-1.12	60	lin	1.048	-1.069E-04	-	-1.11	14	lin	1.045	-9.960E-05	-
	Woody-noN2Fixing	-1.12	201	lin	1.049	-1.076E-04	-	-1.13	65	lin	1.053	-1.170E-04	-
	Woody-N2Fixing	-1.36	22	lin	1.130	-2.832E-04	-	1.31	4	lin	0.870	2.889E-04	-

DLI	Functional groups	Stomatal Density						Stomatal Index					
		PI	n	Regform	a	b	c	PI	n	Regform	a	b	c
	All species	1.93	358	sat	1.476	0.526	0.06731	1.96	127	sat	1.285	0.553	0.11747
	Herbaceous	2.07	175	sat	1.459	0.571	0.09146	1.76	89	sat	1.206	0.497	0.14102
	Woody	1.95	183	sat	1.585	0.536	0.04805	2.54	38	sat	1.480	0.669	0.09477
	Herb-C3-Annuals	2.38	119	sat	1.623	0.631	0.06938	2.06	72	sat	1.909	0.600	0.02910
	Herb-C3-Perennials	1.84	32	sat	1.605	0.507	0.04534	1.79	11	sat	1.098	0.497	0.11293
	Herb-C3	2.27	151	sat	1.607	0.609	0.06813	1.79	83	sat	1.237	0.502	0.12445
	Herb-C4	1.19	21	sat	1.101	0.179	0.10178	-	6	-	-	-	-
	Herb-C3-Monocots	1.62	21	sat	1.288	0.421	0.09073	1.26	11	sat	1.593	0.439	0.00839
	Herb-C3-Dicots	2.37	130	sat	2.169	0.666	0.02787	2.10	72	sat	1.855	0.599	0.03258
	Herb-C3-noN2Fixing	2.18	104	sat	1.418	0.604	0.10887	1.66	64	sat	1.134	0.483	0.19354
	Herb-C3-N2Fixing	2.45	45	sat	15.538	0.949	0.00170	3.47	19	sat	25.308	0.972	0.00158
	Woody	1.95	179	sat	1.583	0.536	0.04847	2.54	38	sat	1.480	0.669	0.09477
	Woody-Deciduous	1.56	39	sat	1.416	0.399	0.04870	2.58	9	sat	1.665	0.665	0.05799
	Woody-Evergreen	2.20	121	sat	1.938	0.624	0.03142	2.52	23	sat	1.467	0.667	0.09663
	Woody-Angiosperms	1.90	176	sat	1.493	0.519	0.05870	2.52	36	sat	1.467	0.667	0.09663
	Woody-Gymnosperms	1.98	5	sat	5.841	0.859	0.00370	-	0	-	-	-	-
	Woody-noN2Fixing	2.06	159	sat	1.706	0.572	0.04142	2.54	38	sat	1.480	0.669	0.09477
	Woody-N2Fixing	1.58	20	sat	1.541	0.444	0.02905	-	0	-	-	-	-

Temperature	Group	PI	n	Regform	a	b	c
	All species	1.54	152	lin	0.710	1.402E-02	-
	Herbaceous	1.37	80	lin	0.790	1.039E-02	-
	Woody	2.00	66	lin	0.553	2.209E-02	-
	Herb-C3-Annuals	1.27	65	lin	0.835	7.886E-03	-
	Herb-C3-Perennials	1.89	10	lin	0.569	1.986E-02	-
	Herb-C3	1.41	75	lin	0.762	1.118E-02	-
	Herb-C4	-1.04	5	lin	1.056	-1.326E-03	-
	Herb-C3-Monocots	1.76	24	lin	0.632	1.823E-02	-
	Herb-C3-Dicots	1.24	51	lin	0.859	7.052E-03	-
	Herb-C3-noN2Fixing	1.58	43	lin	0.701	1.498E-02	-
	Herb-C3-N2Fixing	1.08	32	lin	0.947	2.653E-03	-
	Woody	1.88	50	lin	0.587	2.026E-02	-
	Woody-Deciduous	2.40	41	lin	0.454	2.749E-02	-
	Woody-Evergreen	1.45	9	lin	0.791	1.281E-02	-
	Woody-Angiosperms	2.01	64	lin	0.549	2.224E-02	-
	Woody-Gymnosperms	1.31	2	lin	2.428	-1.103E-01	-
	Woody-noN2Fixing	1.88	50	lin	0.587	2.026E-02	-
	Woody-N2Fixing	-	0	-	-	-	-

PI	n	Regform	a	b	c
-1.37	10	none	1.218	-0.011	-
-2.16	5	none	1.482	-0.024	-
2.48	5	none	0.445	0.029	-
-	1	-	-	-	-
-1.66	2	none	1.390	-0.017	-
-2.22	3	none	1.491	-0.025	-
-17.38	2	none	3.092	-0.084	-
-1.66	2	none	1.390	-0.017	-
-	1	-	-	-	-
-1.66	2	none	1.390	-0.017	-
-	1	-	-	-	-
-	3	-	-	-	-
-	3	-	-	-	-
-	0	-	-	-	-
2.48	5	none	0.445	0.029	-
-	0	none	-	-	-
-	3	none	-	-	-
-	0	none	-	-	-

Water availability	Group	PI	n	Regform	a	b	c
	All species	1.14	106	none	0.871	0.129	-
	Herbaceous	1.13	56	none	0.877	0.123	-
	Woody	1.32	48	none	0.744	0.256	-
	Herb-C3-Annuals	1.14	32	none	0.871	0.129	-
	Herb-C3-Perennials	-1.38	20	none	1.398	-0.398	-
	Herb-C3	1.09	52	none	0.915	0.085	-
	Herb-C4	1.25	4	none	0.786	0.214	-
	Herb-C3-Monocots	1.09	25	none	0.915	0.085	-
	Herb-C3-Dicots	1.13	27	none	0.877	0.123	-
	Herb-C3-noN2Fixing	1.14	45	none	0.871	0.129	-
	Herb-C3-N2Fixing	-1.53	7	none	1.562	-0.562	-
	Woody	1.38	43	none	0.710	0.290	-
	Woody-Deciduous	-1.03	19	none	1.033	-0.033	-
	Woody-Evergreen	1.41	22	none	0.696	0.304	-
	Woody-Angiosperms	1.32	46	none	0.744	0.256	-
	Woody-Gymnosperms	-1.13	2	none	1.134	-0.134	-
	Woody-noN2Fixing	1.41	38	none	0.696	0.304	-
	Woody-N2Fixing	-1.59	5	none	1.626	-0.626	-

PI	n	Regform	a	b	c
1.01	32	none	0.991	0.009	-
1.01	20	none	0.991	0.009	-
1.04	12	none	0.962	0.038	-
1.01	14	none	0.991	0.009	-
1.05	4	none	1.060	0.053	-
-1.05	18	none	1.054	-0.054	-
1.06	2	none	0.941	0.059	-
-1.34	6	none	1.353	-0.353	-
1.12	12	none	0.886	0.114	-
-1.14	16	none	1.143	-0.143	-
1.12	2	none	0.886	0.114	-
1.21	7	none	0.818	0.182	-
1.25	5	none	0.789	0.211	-
-	0	-	-	-	-
1.04	12	none	0.962	0.038	-
-	0	-	-	-	-
1.21	4	none	0.818	0.182	-
1.04	3	none	0.962	0.038	-