METHODS

*STUDY SITE SELECTION*

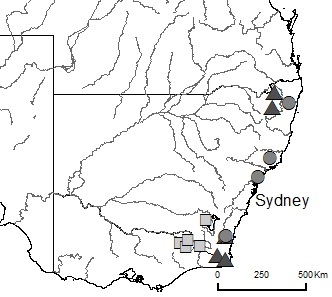
Fifteen riparian sites were selected along gauged rivers within the South-East Coast and south-eastern Murray Darling drainage basins of Australia (Fig. 1 and Table 1). These sites were distributed across clear gradients of ecologically relevant dimensions of hydrological variation: specifically, the magnitude, frequency, duration, timing and rates of change of flow events and patterns.

The following criteria were applied in the site selection process: gauged locations were selected that had >15 years of associated continuous hydrological data, and an absence of flow regulation, significant water extraction or catchment urbanisation, following Kennard et al. (2010). To minimise signals associated with human land-use and river type, the following further criteria were used to shortlist possible study sites: all were partly confined valleys with discontinuous floodplain pocket River Styles, *c.f.* Brierley & Fryirs (2005), had an intact native riparian vegetation cover (a band of native riparian vegetation extending >15 m from the bankfull channel edge), were in good geomorphic condition (lack of significant human-induced erosional or depositional landforms), minimal vegetation clearing (catchment predominantly covered by native vegetation) and occurred in a catchment smaller than 1000 km2. These criteria were assessed using a combination of visual inspection of satellite photography (Google Earth, Microsoft Bing) and information from the NSW Riparian Vegetation Extent dataset and the NSW Office of Water River Styles® geospatial dataset (Healy, Raine, Parsons, & Cook, 2012). To select the 15 study sites from this shortlist, accessibility by road, permission from state or private landholders, and proximity of accessible areas to continuous hydrological monitoring stations were taken into account.

The resulting study area spanned latitude -29.467 to -37.371 and longitude 147.413 to 152.217, and incorporated two Koppen climate zones: temperate, without dry season, hot summer (Cfa) and temperate, without dry season, warm summer (Cfb) (Peel et al. 2007). Sites spanned an altitudinal range of 23 – 732 m asl.

**MAP**

**Figures**

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***Figure 1.*** *Location of fifteen field study sites across south-eastern Australia chosen to represent the three major hydrological classes of south-east Australia. Hydrological class membership is denoted by: • stable winter baseflow, ▲ unpredictable baseflow, ■ unpredictable intermittent. Note that the points representing the two southern-most unpredictable baseflow sites are overlapping.*

**SITE TABLE**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gauge Name | Longitude | Latitude | Catchment area (Km2) | Elevation |
| Snowy Creek at Below Granite Flat | 147.413 | -36.569 | 416 | 331 |
| Gibbo River at Gibbo Park | 147.709 | -36.756 | 390 | 515 |
| Nariel Creek at Upper Nariel | 147.826 | -36.444 | 261 | 711 |
| Goodradigbee River at Brindabella | 148.731 | -35.421 | 432 | 510 |
| Jacobs River at Jacobs Ladder | 148.427 | -36.727 | 184 | 343 |
| Tuross River at Belowra | 149.709 | -36.201 | 564 | 105 |
| Genoa River at Bondi | 149.321 | -37.174 | 234 | 417 |
| Wallagaraugh River at Princes Highway | 149.714 | -37.371 | 477 | 35 |
| Mann River at Mitchell | 152.105 | -29.695 | 890 | 401 |
| Cataract Creek at Sandy Hill | 152.217 | -28.934 | 237 | 595 |
| Jilliby Creek at U/S Wyong River | 151.389 | -33.246 | 93 | 39 |
| Sportsmans Creek at Gurranang Siding | 152.981 | -29.467 | 205 | 13 |
| Mammy Johnsons River at Pikes Crossing | 151.979 | -32.244 | 158 | 104 |
| Wadbilliga River at Wadbilliga | 149.694 | -36.259 | 126 | 201 |
| Tuross River D/S Wadbilliga Junction | 149.761 | -36.197 | 918 | 79 |

VEGETATION SURVEYS

Vegetation surveys were undertaken between December 2012 and May 2013. At each site, a 10 m by 50 m plot was marked out, with the longest edge abutting the channel edge. Criteria for selection of plot locations were: geomorphic homogeneity (the plot comprising only gently sloping bank where possible), maximum 2m elevational difference between lower and upper edge of plot, and lack of anthropogenic disturbance such as built structures, roads or tracks, recent logging or clearing (in the last 20-30 years), herbicide spraying or animal grazing.

Proportional cover of woody vegetation was assessed for three strata: shrub (1-4 m), sub canopy (4-8 m) and canopy (>8 m). Species within plots were identified using appropriate field guides, and field identifications were verified against herbarium specimens at the Macquarie University Herbarium. Some specimens were identified by staff at the Royal Botanic Gardens, Sydney.

TRAIT DATA COLLECTION

*Rationale for trait selection*

Data for the following traits were collected: maximum canopy height, seed mass, specific leaf area (SLA), wood density, flowering period (as proportion of the year), leaf narrowness (the ratio of leaf width to length). These traits were chosen to encapsulate the key axes of variation relevant to ecological strategies employed by riparian plants. Variation throughout this constellation of traits should, then, provide a relevant indication of functionally diversity for riparian communities.

Specific leaf area, maximum canopy height and seed mass comprise the LHS (leaf, height, seed) triad of traits introduced by Westoby et al. (1998) as a general scheme for comparing the properties of vegetation communities. These three traits are typically distributed orthogonally from each other and represent fundamental trade-offs that control plant ecological strategy (Westoby et al. 1998). LEPS ET AL SAID DIAZ ET AL SAID LHS IS ENOUGH

Seed mass indicates maternal investment in offspring and is a fundamental determinant of seedling establishment success (Leishman et al. 2000); time to reproduction may offset this initial advantage, however (Moles & Westoby 2006). In the riparian environment, seed mass may therefore be constrained if repeated flooding disturbance excludes species with long generation times.

Maximum canopy height integrates the central trade-off between competition for light, and construction and maintenance of costly support structures such as woody stems (REFS – Westoby 1998 not enough). These costs are particularly accentuated where plants are must defend stems from mechanical disturbance (REF?).

Specific leaf area is a useful indicator of a species’ position along the leaf economics spectrum. High SLA species invest considerable nutrients in their leaves, have high rates of photosynthesis and respiration, and short leaf longevity; these species typically exhibit high relative growth rates. Conversely, low SLA species receive slower return on investment on costly leaves, with lower rates of photosynthesis and respiration, but greater leaf longevity and ability to tolerate stressful conditions (Wright et al. 2004; Reich & Wright 2003).

Wood density, defined as oven dry mass divided by green volume, is an emergent property that integrates a number of anatomical traits of lignified tissues (Chave et al. 2009). Dense wood is costly to construct and has been linked with slower relative growth rates, but increased cohort survivorship (e.g. King *et al.* 2006; Poorter *et al.* 2008, 2010; Kraft *et al.* 2010; Wright *et al.* 2010). Wood density has also been associated with environmental stress tolerance (Martínez-Cabrera et al. 2009; Preston, Cornwell & Denoyer 2006) and enhanced resistance to disturbance (Lawson et al. 2014; Curran *et al.* 2008, Telewski 1995). These latter two associations are significant in riparian environments, where flooding disturbance and pulsed periods of water stress are often commonplace (REF).

Patterns of seasonality across south eastern Australia are variable spatially as well as temporally, owing to complexs interaction between geography and decadal-scale oscillations in climate, such as the Pacific Decadal Oscillation and El Nino Southern Oscillation. The lack of a strong period of cold-induced dormancy in this region means plants may flower at any time of the year. Flowering period was used here as an indicator species’ ability to respond reproductively to favourable conditions.

Leaf narrowness provides two-fold information about plant ecological strategy. For one, narrow leaves are able to regulate temperature more efficiently and thus maintain photosynthesis hot in or highly insolated (i.e. disturbed) conditions (Parkhurst and Loucks 1972; Givnish 1987; Fonseca et al. 2000). Leaf narrowness is also strongly indicative of rheophyty – the trait syndrome shared by plants adapted to growing near swift flowing, intensely flooded streams (Van Steenis 1950 something).

*Dataset assembly*

The dataset for this study was assembled using measurements recorded in the field (specific leaf area, wood density), supplemented by data from published literature, private and public trait databases and Australian flora texts; see *Appendix 2* a detailed bibliography of references for data. Where multiple values were found for a single site, values were excluded if they were measured from sites that were substantially different from the environmental conditions of the field site they were found in. Remaining values were averaged. Single values for each trait were recorded, under the assumption that intraspecific variability is less than interspecific variability (REF – Westoby 1998, plus more? – Michaels et al 1988 for seed size). Leaf narrowness was not included for grasses, seed mass and flowering period were not included for ferns.

Wood samples for wood density measurement were collected from dominant woody species present within each plot at >5% cover in shrub, sub canopy or canopy strata, and which had trunks robust enough to core (typically > 5 cm diameter at base). A 100 mm wood sample from each of two individuals per species was extracted using a 5.15 mm diameter, triple threaded increment borer (Haglöf Sweden). Samples were extracted from the base of the main trunk, 10 cm above the leaf litter level, and air-dried at 20-45 °C. On return to the laboratory, samples were rehydrated in deionised water and 10 mm sections of mature wood were cut with a razor, using visual inspection of vessel occlusion as an indicator of maturity. Sections were measured (x, y and z dimensions) with callipers (Mitutoyo America, Illinois USA) to calculate wet volume, then oven-dried at 80°C for 48 hours and weighed using a microbalance (Mettler Toledo, Greifensee, Switzerland). Wood density was then calculated as the ratio of oven dry mass to wet volume (g/cm3). Site-specific values were thus available for wood density (due collection for a concurrent project focusing on woody tissues), but for the purposes of this study, an overall mean value was calculated for species which occurred at multiple sites. Species for which data could not be obtained in the field were assigned values from the Global Wood Density Database (Chave et al. 2009).

SLA was measured once for each species according to the procedure defined by Cornellisen (2003). A minimum of five new, but fully mature leaves from well-lit areas were taken from each of five non-contiguous individuals. Leaves were pressed in the field to maintain fresh area and allowed to air dry 20-45 °C, then scanned and leaf area measurements made using image analysis software (ImageJ 1.48 for Windows). Leaves were then oven dried at 60 °C for 72 hours and weighed using a microbalance (Mettler Toledo, Greifensee, Switzerland). SLA was then calculated as area divided by dry mass.

HYDROLOGICAL ANALYSIS

Daily discharge data for each of the 15 sites were taken with permission from Lawson et al. (2014, in review). Hydrological analysis was identical to Lawson et al. (2014), with the inclusion of a further 8 metrics describing flow seasonality; the reader is referred to this publication for a full description of analysis methods. We generated set of 33 hydrological metrics for each site, based on a reduction of the minimally redundant set of ecologically relevant metrics for Australian rivers described by Kennard et al. (2010). These metrics were chosen as descriptors of the frequency and magnitude of flooding disturbance, as well as variability in water availability across seasons and between years (see **Table X** for descriptions of individual metrics). Metrics of flow magnitude which had units ML / day were normalised by mean daily flow to allow for comparison between different sizes of river.

*Table 3 – descriptions*

*Table X – summary statistics for hydrological metrics*

| **metric** | **min** | **max** | **mean** | **sd** |
| --- | --- | --- | --- | --- |
| HSPeak | 5.38 | 29.81 | 16.67 | 8.34 |
| MDFAnnHSNum | 2.80 | 5.93 | 4.10 | 0.96 |
| CVAnnHSNum | 0.48 | 0.84 | 0.74 | 0.11 |
| CVAnnHSPeak | 0.24 | 1.47 | 0.69 | 0.34 |
| MRateRise | 0.20 | 1.99 | 0.91 | 0.57 |
| MRateFall | 0.07 | 0.80 | 0.34 | 0.23 |
| CVAnnMRateRise | 0.43 | 1.18 | 0.85 | 0.25 |
| CVAnnMRateFall | 0.41 | 1.46 | 0.90 | 0.34 |
| AS20YrARI | 17.94 | 209.99 | 126.13 | 81.19 |
| C\_MDFM | 0.05 | 0.31 | 0.14 | 0.09 |
| M\_MDFM | 0.06 | 0.20 | 0.12 | 0.05 |
| C\_MinM | 0.01 | 0.27 | 0.12 | 0.08 |
| M\_MinM | 0.07 | 0.16 | 0.11 | 0.03 |
| C\_MaxM | 0.19 | 0.44 | 0.28 | 0.09 |
| M\_MaxM | 0.04 | 0.18 | 0.09 | 0.06 |
| MDFMDFSpring | 0.19 | 1.81 | 1.02 | 0.55 |
| MDFMDFSummer | 0.42 | 1.49 | 0.88 | 0.33 |
| MDFMDFAutumn | 0.28 | 1.82 | 1.00 | 0.52 |
| MDFMDFWinter | 0.64 | 1.44 | 1.08 | 0.25 |
| CVMDFSpring | 0.36 | 2.10 | 1.12 | 0.54 |
| CVMDFSummer | 0.60 | 1.66 | 1.15 | 0.39 |
| CVMDFAutumn | 0.48 | 1.49 | 1.07 | 0.35 |
| CVMDFWinter | 0.46 | 1.99 | 1.05 | 0.46 |
| Latitude (o) | -28.93 | -37.37 | -34.58 | -3.04 |
| Catchment area (km2) | 93.00 | 918.00 | 372.33 | 255.95 |
| Elevation (m asl) | 13.00 | 711.00 | 293.27 | 228.05 |

FUNCTIONAL DIVERSITY ANALYSIS

All statistical analyses were performed using the R statistical programming environment (R Core Team, 2013). The R code used for these analyses can be retrieved from <https://github.com/jamesrlawson/functional-diversity/tree/master/scripts>. Statistical significance was interpreted at alpha = 0.05.

*Functional diversity*

Only species present at >1 % cover in plots were included in the analysis (n=107, from a total of 327 species). Sufficient trait data were available for inclusion in the analysis for 107 species of a total 126 species identified as present at > 1% abundance (see Table X for data density information). Data deficient species lacking values for more than four traits could not be included as they produced gaps in the distance matrix. All trait values were transformed by log10 prior to analysis.

Table X – Data density. N.B. leaf narrowness and wood density were not available for grasses or ferns; seed mass and flowering period were also not available for ferns.

|  | **plotID** | **coverage** | **WD** | **maxheight** | **seedmass** | **SLA** | **flowering.period** | **length.width.ratio** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **1** | 1 | 0.980 | 0.615 | 1.00 | 0.846 | 1.000 | 0.923 | 0.692 |
| **2** | 2 | 0.959 | 0.333 | 1.00 | 0.667 | 1.000 | 0.667 | 0.333 |
| **3** | 3 | 0.949 | 0.455 | 1.00 | 0.727 | 1.000 | 0.727 | 0.545 |
| **4** | 4 | 0.903 | 0.400 | 1.00 | 0.867 | 1.000 | 0.867 | 0.600 |
| **5** | 5 | 0.968 | 0.455 | 1.00 | 1.000 | 1.000 | 1.000 | 0.545 |
| **6** | 6 | 0.964 | 0.700 | 1.00 | 1.000 | 1.000 | 1.000 | 0.700 |
| **7** | 7 | 1.000 | 0.500 | 1.00 | 1.000 | 0.900 | 1.000 | 0.700 |
| **8** | 8 | 1.000 | 0.529 | 1.00 | 0.882 | 1.000 | 0.882 | 0.765 |
| **9** | 9 | 0.988 | 0.474 | 1.00 | 0.842 | 1.000 | 0.842 | 0.737 |
| **10** | 10 | 0.976 | 0.583 | 1.00 | 0.917 | 1.000 | 0.917 | 0.667 |
| **11** | 11 | 0.960 | 0.188 | 1.00 | 1.000 | 0.938 | 1.000 | 0.688 |
| **12** | 12 | 0.992 | 0.381 | 1.00 | 0.952 | 0.952 | 0.952 | 0.714 |
| **13** | 13 | 0.935 | 0.550 | 0.95 | 0.900 | 1.000 | 0.900 | 0.700 |
| **14** | 14 | 1.000 | 0.636 | 1.00 | 1.000 | 1.000 | 1.000 | 1.000 |
| **15** | 15 | 0.963 | 0.455 | 1.00 | 0.909 | 0.909 | 0.909 | 0.727 |

Table x – summary statistics for traits

| **variable** | **min** | **max** | **mean** | **sd** |
| --- | --- | --- | --- | --- |
| Maxheight (m) | 0.20 | 50.00 | 10.47 | 13.18 |
| Seedmass (mg) | 0.04 | 323.99 | 16.55 | 45.06 |
| SLA (m2 / kg) | 1.41 | 63.27 | 17.93 | 14.00 |
| Flowering period (proportion of year) | 0.17 | 1.00 | 0.45 | 0.24 |
| Leaf narrowness (unitless ratio) | 0.59 | 233.33 | 9.86 | 32.53 |
| Wood density (g / cm3) | 0.33 | 0.95 | 0.61 | 0.13 |

On the advice of Leps et al. (2006), we performed principal components analysis (PCA) (stats package, R Core Team 2013) on trait data to check for redundancy. Although not completely orthogonal, traits were distributed across multiple principal components (see Table Xa,b for loadings). Therefore we believe there is both ecological (as previously discussed) and statistical rationale to retain all six traits in the analysis.

Table Xa – traits.woody

> summary(traits.woody.PCA)

Importance of components:

PC1 PC2 PC3 PC4 PC5 PC6

Standard deviation 1.3938 1.0962 1.0827 0.9247 0.7438 0.52457

Proportion of Variance 0.3238 0.2003 0.1954 0.1425 0.0922 0.04586

Cumulative Proportion 0.3238 0.5240 0.7194 0.8619 0.9541 1.00000

PC1 PC2 PC3 PC4 PC5 PC6

maxheight 0.3573963 -0.1987059 -0.66583256 -0.11435121 0.5016529 -0.353155880

seedmass -0.1798937 0.3943905 -0.69938677 0.06524450 -0.5545229 0.105830448

SLA -0.6371510 -0.2168621 0.02160485 0.02968953 -0.1106811 -0.730349857

flowering.period -0.2621872 0.5266711 0.05707758 -0.74907001 0.2991799 -0.001755496

length.width.ratio 0.5314203 -0.1041159 0.15870620 -0.47444131 -0.5712787 -0.360708643

WD 0.2877415 0.6853615 0.19650159 0.44224998 0.1128292 -0.447834925

Table Xb – traits.naomit

> summary(traits.minimal.PCA)

Importance of components:

PC1 PC2 PC3 PC4

Standard deviation 1.4160 1.0016 0.8326 0.54649

Proportion of Variance 0.5012 0.2508 0.1733 0.07466

Cumulative Proportion 0.5012 0.7520 0.9253 1.00000

PC1 PC2 PC3 PC4

maxheight -0.6320275 0.07961338 -0.1966850 0.74533073

seedmass -0.2943732 0.83071568 0.4131076 -0.22934247

SLA 0.5873879 0.12689057 0.5066191 0.61823234

flowering.period 0.4109270 0.53616411 -0.7307490 0.09835091

We used the *dbFD* function from the FD package (Laliberte & Legendre 2010) to calculate functional dispersion (FDis). This package implements the method for distance-based tests for homogeneity of multivariate dispersions described by Anderson (2006). *dbFD* useds Gower’s method (1971) to generate the dissimilarity matrix, which can account for missing values, and automatically standardises traits by their ranges; Cailliez’s correction was applied to the matrix.

*Regression models*

Ordinary least-squares (OLS) regression models were generated for selected metrics to determine relationships between hydrological gradients and FDis. To reduce the occurrence of Type 1 statistical error, we adjusted the resulting p values using the two step Benjamini - Hochberg (BH) procedure (Benjamini, Krieger, & Yekutieli, 2006) for controlling the false discovery rate (*mt.rawp2adjp* function in multtest package) (Pollard, Ge, & Dudoit, 2008). This two step BH method has been shown to control the false discovery rate for positively dependent test statistics, and provides a better estimate of the false discovery rate than the original BH algorithm (Benjamini & Hochberg, 1995) by adaptively controlling p value adjustment according to the number of true null hypotheses obtained from the first pass of the procedure.

To investigate the influence of regional environmental variables on functional diversity, we used OLS regression to compare FDis with latitude, elevation above sea level and catchment area. We also tested the relationships between FDis and species richness and Simpson diversity (for species used in the analysis, present at > 1 % cover), and species richness for the full set of 327 species identified in the study.

We selected a minimal multiple regression model designed to incorporate descriptors of disturbance frequency and magnitude, and flow variability. The full set of hydrological metrics was initially screened to remove metrics which were individually determined to have non-significant relationships with FDis, although metrics which were rendered non-significant by BH adjustment were included. PCA over the selected metrics identified one major and two minor axes of variation (PC1 – 71.4 %, PC2 – 9.0 % and PC3 - 8.3 % of variance explained). For PC1 there was no clear differentiation in eigenvalues; the used metric with highest individual R2 value (CVAnnHSPeak) was selected. PC2 identified MDFMDFSummer and PC3 identified CVAnnHSNum as further sources of variability. Models were then built pertaining to all possible permutations of summation and interaction for these three metrics. Values for each metric were centred by subtracting the mean value (after Robinson & Schumacker, 2009). Multicollinearity was tested for according to the variance inflation factor (VIF) score (HH package, Heiberger & Holland, 2004), and models were compared according the second order of Akaike’s Information Criterion (AIC) (MuMIn package, Barton, 2012). Second order AIC is recommended in order to reduce bias when the ratio of sample size to number of predictor variables is small (Burnham & Anderson, 2002).