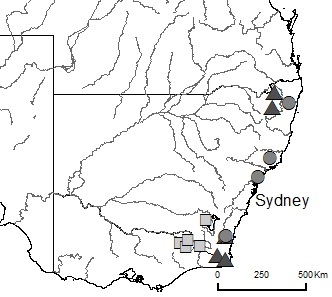
METHODS

*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 *et al.* 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.Sites spanned an altitudinal range of 23 – 732 m above sea level.

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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.*

Table 1. Location and characteristics of field sites.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Site # | Gauge Name | Longitude | Latitude | Catchment area (Km2) | Elevation |
| 1 | Mammy Johnsons River at Pikes Crossing | 151.979 | -32.244 | 158 | 104 |
| 2 | Wallagaraugh River at Princes Highway | 149.714 | -37.371 | 477 | 35 |
| 3 | Genoa River at Bondi | 149.321 | -37.174 | 234 | 417 |
| 4 | Wadbilliga River at Wadbilliga | 149.694 | -36.259 | 126 | 201 |
| 5 | Tuross River D/S Wadbilliga Junction | 149.761 | -36.197 | 918 | 79 |
| 6 | Tuross River at Belowra | 149.709 | -36.201 | 564 | 105 |
| 7 | Jacobs River at Jacobs Ladder | 148.427 | -36.727 | 184 | 343 |
| 8 | Nariel Creek at Upper Nariel | 147.826 | -36.444 | 261 | 711 |
| 9 | Gibbo River at Gibbo Park | 147.709 | -36.756 | 390 | 515 |
| 10 | Snowy Creek at Below Granite Flat | 147.413 | -36.569 | 416 | 331 |
| 11 | Mann River at Mitchell | 152.105 | -29.695 | 890 | 401 |
| 12 | Cataract Creek at Sandy Hill | 152.217 | -28.934 | 237 | 595 |
| 13 | Sportsmans Creek at Gurranang Siding | 152.981 | -29.467 | 205 | 13 |
| 14 | Goodradigbee River at Brindabella | 148.731 | -35.421 | 432 | 510 |
| 15 | Jilliby Creek at U/S Wyong River | 151.389 | -33.246 | 93 | 39 |

*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.

*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, (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).

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 (Westoby 1998). These costs are particularly accentuated where plants are must defend stems from mechanical disturbance (Falster 2006).

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 (Reich & Wright 2003; Wright *et al.* 2004).

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, Davies, Tan, & Noor, 2006; Kraft, Metz, Condit, & Chave, 2010; Poorter et al., 2008; S. J. Wright et al., 2010). Wood density has also been associated with environmental stress tolerance (Preston, Cornwell & Denoyer 2006; Martínez-Cabrera *et al.* 2009) and enhanced resistance to disturbance (Telewski 1995; Curran *et al.* 2008)(+Lawson et al. 2014). These latter two associations are significant in riparian environments, where flooding disturbance and pulsed periods of water stress are commonplace.

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 (Cornelissen *et al.* 2003). 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 1981).

*Trait 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 (Michaels, Benner & Hartgerink 1988; Westoby 1998). 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 to the nearest 0.01 mm (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); the reader is referred to this publication for a full description of analysis methods. We generated set of 23 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 2for descriptions of individual metrics). Summary statistics for hydrological metrics are shown in Table 3. Metrics of flow magnitude which had units ML / day were standardised by mean daily flow to allow for comparison between different sizes of river. These metrics therefore represent ratios of flow magnitude to mean daily flow.

Table 2. Hydrological parameters used as metrics of frequency and magnitude of flooding disturbance and variability in seasonal water availabily in the riparian zone.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **Abbreviation** | | **Units** | **Description** |
| *Flood frequency and magnitude* | | | | |
| Mean magnitude of high spells\* | HSPeak | dimensionless | | High spells are periods of flow above the 95th percentile on the flow duration curve. We were interested in how frequently these conditions occurred over the time series as well as the mean magnitude of peak flows during these periods. 20 year average return interval (ARI) floods are extreme flow events that have the potential to re-work the fluvial landscape. Together, these metrics indicate the intensity and frequency of mechanical stress experienced by plants in the riparian zone. |
| CV of all years’ mean high spell magnitude | CVAnnHSPeak | dimensionless | |
| 20 year ARI flood magnitude\* | AS20YrARI | dimensionless | |
| Mean of all years’ number of high spells | MDFAnnHSNum | year-1 | |
| CV of all years’ number of high spells | CVAnnHSNum | dimensionless | |
| *Rise and fall rates* | | | | |
| Mean rate of rise \* | MRateRise | day-1 | | Rise and fall rates represent flow ‘flashiness’. Fast rise rates are associated with flood waves and entrainment of debris into the flood channel. Slow fall rates keep exposed substrate moist for longer periods, which may produce favourable conditions for germination. Historical discharge records are unfortunately limited to daily resolution, so are unable to fully capture flood discharge shapes. |
| Mean rate of fall \* | MRateFall | day-1 | |
| CV of all years’ mean rate of rise | CVAnnMRateRise | dimensionless | |
| CV of all years’ mean rate of fall | CVAnnMRateFall | dimensionless | |
| *Colwell’s indices* | | | | |
| Constancy of monthly mean daily flow | C\_MDFM | dimensionless | | Colwell’s indices provide a measure of the seasonal predictability of flow events and therefore water availability within the riparian zone. Constancy (C) measures uniformity of flow across seasons, and is maximised when flow conditions do not differ between seasons. Contingency (M) is a measure of interannual uniformity in seasonal flow patterns, and is maximized when seasonal patterns of flow are consistent between years.  We generated Colwell’s indices for both average flow conditions and minimum flows conditions. |
| Contingency of monthly mean daily flow | M\_MDFM | dimensionless | |
| Constancy based on monthly minimum daily flow | C\_MinM | dimensionless | |
| Contingency based on monthly minimum daily flow | M\_MinM | dimensionless | |
| *Flow seasonality* | | | | |
| Average mean daily flow for Spring \* | MDFMDFSpring | dimensionless | | These metrics describe the average magnitude and variability within mean daily flows for each season. Averages and coefficients of variation are calculated across yearly means. Seasonal average mean daily flows were standardised by overall mean daily flow, so actually represent the ratio of mean daily flow in a given season to the total mean daily flow. |
| Average mean daily flow for Summer \* | MDFMDFSummer | dimensionless | |
| Average mean daily flow for Autumn \* | MDFMDFAutumn | dimensionless | |
| Average mean daily flow for Winter \* | MDFMDFWinter | dimensionless | |
| CV of mean daily flow for Spring | CVMDFSpring | dimensionless | |
| CV of mean daily flow for Summer | CVMDFSummer | dimensionless | |
| CV of mean daily flow for Autumn | CVMDFAutumn | dimensionless | |
| CV of mean daily flow for Winter | CVMDFWinter | dimensionless | |

Table 3. Summary statistics for hydrological and miscellaneous environmental variables.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| metric | min | max | mean | sd |
| HSPeak | 5.38 | 29.81 | 16.67 | 8.34 |
| MDFAnnHSNum | 2.8 | 5.93 | 4.1 | 0.96 |
| CVAnnHSNum | 0.48 | 0.84 | 0.74 | 0.11 |
| CVAnnHSPeak | 0.24 | 1.47 | 0.69 | 0.34 |
| MRateRise | 0.2 | 1.99 | 0.91 | 0.57 |
| MRateFall | 0.07 | 0.8 | 0.34 | 0.23 |
| CVAnnMRateRise | 0.43 | 1.18 | 0.85 | 0.25 |
| CVAnnMRateFall | 0.41 | 1.46 | 0.9 | 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 | 0.2 | 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 | 0.52 |
| MDFMDFWinter | 0.64 | 1.44 | 1.08 | 0.25 |
| CVMDFSpring | 0.36 | 2.1 | 1.12 | 0.54 |
| CVMDFSummer | 0.6 | 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 | 918 | 372.33 | 255.95 |
| Elevation (m asl) | 13 | 711 | 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.

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 4 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 5 gives summary statistics for the trait dataset.

Table 4. Data density. Coverage describes the total proportional coverage at a site for which species were included in the analysis. Density values for each trait describe the proportional coverage at a site for which data about that trait was included in the analysis. 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.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| site # | coverage | wood density | max. height | seed mass | SLA | flowering period | leaf narrowness |
| 1 | 0.98 | 0.615 | 1 | 0.846 | 1 | 0.923 | 0.692 |
| 2 | 0.959 | 0.333 | 1 | 0.667 | 1 | 0.667 | 0.333 |
| 3 | 0.949 | 0.455 | 1 | 0.727 | 1 | 0.727 | 0.545 |
| 4 | 0.903 | 0.4 | 1 | 0.867 | 1 | 0.867 | 0.6 |
| 5 | 0.968 | 0.455 | 1 | 1 | 1 | 1 | 0.545 |
| 6 | 0.964 | 0.7 | 1 | 1 | 1 | 1 | 0.7 |
| 7 | 1 | 0.5 | 1 | 1 | 0.9 | 1 | 0.7 |
| 8 | 1 | 0.529 | 1 | 0.882 | 1 | 0.882 | 0.765 |
| 9 | 0.988 | 0.474 | 1 | 0.842 | 1 | 0.842 | 0.737 |
| 10 | 0.976 | 0.583 | 1 | 0.917 | 1 | 0.917 | 0.667 |
| 11 | 0.96 | 0.188 | 1 | 1 | 0.938 | 1 | 0.688 |
| 12 | 0.992 | 0.381 | 1 | 0.952 | 0.952 | 0.952 | 0.714 |
| 13 | 0.935 | 0.55 | 0.95 | 0.9 | 1 | 0.9 | 0.7 |
| 14 | 1 | 0.636 | 1 | 1 | 1 | 1 | 1 |
| 15 | 0.963 | 0.455 | 1 | 0.909 | 0.909 | 0.909 | 0.727 |

Table 5. Summary statistics for trait dataset.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| trait | min | max | mean | sd |
| Max. height (m) | 0.2 | 50 | 10.47 | 13.18 |
| Seed mass (mg) | 0.04 | 323.99 | 16.55 | 45.06 |
| SLA (m2 / kg) | 1.41 | 63.27 | 17.93 | 14 |
| Flowering period (proportion of year) | 0.17 | 1 | 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, Bello, Lavorel, & Berman, (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 well distributed across multiple principal components. Therefore we believe there is both ecological (as previously discussed) and statistical rationale to retain all six traits in the analysis.

We used the *dbFD* function from the FD package (Laliberté & 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* uses 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 (Cailliez 1983). Simpson’s diversity was calculated using the SYNCSA package (Debastiani & Pillar 2012).

*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 metric with highest individual R2 value (interannual variability in high flows) was selected. PC2 identified mean daily flow in summer and PC3 identified interannual variability in flood frequency 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).