

Flow regime alteration degrades ecological networks in riparian ecosystems

Jonathan D. Tonkin^{1*}, David. M. Merritt², Julian D. Olden³, Lindsay V. Reynolds¹ and David A. Lytle¹

Riverine ecosystems are governed by patterns of temporal variation in river flows. This dynamism will change due to climate change and the near-ubiquitous human control of river flows globally, which may have severe effects on species distributions and interactions. We employed a combination of population modelling and network theory to explore the consequences of possible flow regime futures on riparian plant communities, including scenarios of increased drought, flooding and flow homogenization (removal of flow variability). We found that even slight modifications to the historic natural flow regime had significant consequences for the structure of riparian plant networks. Networks of emergent interactions between plant guilds were most connected at the natural flow regime and became simplified with increasing flow alteration. The most influential component of flow alteration was flood reduction, with drought and flow homogenization both having greater simplifying community-wide consequences than increased flooding. These findings suggest that maintaining floods under future climates will be needed to overcome the negative long-term consequences of flow modification on riverine ecosystems.

The fundamental human demand for fresh water has resulted in the dramatic transformation of rivers throughout the world¹. Dams, diversions and numerous other forms of infrastructure have enabled water flows to be controlled for human use, resulting in the dampening or elimination of flooding and other ecologically important aspects of flow regimes^{2,3} (see Table 1 for the definition of the natural flow regime). Such effects threaten riparian ecosystems^{4,5} and compromise many functions and services supported by these ecosystems, including the regulation of thermal regimes and water quality, flood attenuation, contribution of carbon to aquatic food webs, and the provision of wood and physical habitat structure along the terrestrial-aquatic interface⁶. These stressors to riparian ecosystems are exacerbated by climate change, which threatens to increase flow intermittency⁷ and modify species distributions and interactions via drought intensification in many regions globally^{8,9}. What has emerged is a clear need for robust forecasting of how riparian ecosystems will respond to changing flooding and drought conditions over long timescales¹⁰.

Riparian ecosystems have evolved in the context of naturally recurring cycles of flooding and drought¹¹. Thus, alterations to species interactions caused by novel climate and flow regimes are feared to contribute to the collapse of previously robust and resilient communities¹². Ecological network theory provides new opportunities to achieve a deeper, more holistic understanding of how organisms interact with each other and their environments¹³. However, while the effects of altered network structure on the functioning of ecosystems has been well explored^{14,15}, we know less about the effects of environmental change on the structure of ecological networks¹⁶ (but see ref. ¹⁷). Despite the potential of network theory to enhance our understanding of how communities are structured, there remains a disconnect between modelling the dynamics of entire communities and the relative roles of individual species or functional groupings of species in these networks¹⁸,

and how these roles vary through space and time with various scenarios of environmental change¹⁶.

Here, we quantify the community-wide consequences of changing flooding and drought regimes on riparian plant communities. By focusing on five riparian plant guilds (groupings of species with similar life histories and vital rates¹⁹) that constitute riparian assemblages across a wide variety of rivers worldwide, our investigation occurs in the western US but is emblematic of dominant trait syndromes in dryland regions. These guilds encompass hydroriparian pioneer trees (HT), xeroriparian pioneer shrubs (XS), hydroriparian pioneer shrubs (HS), mesoriparian meadow species (MM) and desert shrubs (DS) (Table 1). Using these dryland riparian plants with empirically derived biotic information for six stage classes (Fig. 1), we employ a modelling framework based on stochastic, coupled structured population models¹⁰. The model uses fundamental vital rates to explore ecological futures across a spectrum of possible flow regimes for a snowmelt-driven river in the western US, a region predicted to have a future with reduced runoff and altered flow regimes under climate change²⁰. By combining stage-specific responses of plant guilds with specific attributes of a river hydrologic regime, the model enables a mechanistic approach to understanding the non-stationary effects of climate- and human-driven changes to the flow regime on riparian communities.

We employed network theory as a way to visualize and quantify these consequences, as it provides opportunities to understand how groups of organisms interact with each other under different environmental contexts and allows the exploration of how effects propagate throughout complex systems¹³. Here, riparian plant networks were constructed from models, where we quantified interaction strengths and formed network links by using a new form of multi-guild sensitivity analysis (also termed sensitivity networks¹⁰; Supplementary Fig. 1). In these networks, nodes represent life-history stages of different plant guilds, and links between two nodes

¹Department of Integrative Biology, Oregon State University, Corvallis, OR 97331, USA. ²Watershed, Fisheries, Wildlife, Air and Rare Plants Staff and the National Stream and Aquatic Ecology Center, U.S.D.A. Forest Service, Fort Collins, CO 80526, USA. ³School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98195, USA. *e-mail: jdttonkin@gmail.com

Table 1 | A glossary of important terms

Category	Term	Definition
General terms		
	Changepoint	A location in the data where the statistical properties of a sequence of observations change.
	The natural flow regime	The recorded historical pattern of floods and drought, characterized by timing, frequency, magnitude, duration and interannual variability of flows ¹ .
Plant guilds		
	Desert shrub	Drought-tolerant, flood-intolerant, upland shrub (such as woody <i>Artemesia</i> species).
	Hydroriparian pioneer shrub	Flood-adapted, drought-intolerant, phreatophytic species with shrub growth forms and flexible stems that have high water requirements and exist relatively close to the stream channel with similar recruitment requirements to a hydroriparian pioneer tree (such as shrub <i>Salix</i> species).
	Hydroriparian pioneer tree	Long-lived, flood-tolerant, phreatophytic tree species that recruit onto bare, moist substrate near the stream channel following floods (such as <i>Populus</i> , <i>Alnus</i> and <i>Eucalyptus</i> species).
	Mesoriparian meadow species	Facultative phreatophytic species with less-restrictive water requirements and tolerance of exposure to brief drought conditions (such as wetland grass and forb species).
	Xeroriparian pioneer shrub	Drought-tolerant, facultative phreatophytic plant species that require some flooding for regeneration but whose adults can tolerate periods of extended drought (such as <i>Tamarix</i> species).
Networks		
	Degree	The number of links connected to the node. These can be broken down into the number of inbound, outbound and mutual links.
	Keystone node	Nodes that had large effects on the overall network, shown by substantial links out to other nodes. Changes in vital rates of these keystone nodes affected a disproportionate number of other nodes in the network.
	Mutual node	Nodes that played a key reciprocal role in the network by representing important bidirectional associations akin to compensatory dynamics.
	Network	A collection of connected objects (nodes). Connections are formed by links.
	Network connectance	The ratio of the number of links in the network to the total number of possible links.
	Network link (edge)	A connection between each of the nodes. Links represent emergent competition for space in the riparian landscape. Rather than in situ observed interactions, these represent emergent interactions between guilds, arising from the model results, through a shared dependency on finite space. We employed sensitivity analysis to infer interaction strengths within and among guilds, and used these values to build a competitive network (see Methods for details). Networks were therefore not defined a priori, but arose from population dynamics.
	Network node (vertex)	An object in the network. Here a node is a single stage of a given guild. There are 6 stages for each of the 5 guilds (30 nodes in total).
	Network reciprocity	The proportion of mutual or two-way links, where one node affects another node and is affected in return.
	Network robustness	A measure of the tolerance of a network to perturbations, such as extinctions. This is commonly measured by examining the network-wide effects of node deletion.
	Passive node	Nodes that had more links in than out and thus responded to, rather than influenced, other nodes in the network.

are formed based on model sensitivity analysis; that is, a link is drawn when a small change in the vital rate of one node (in this case, flood susceptibility) significantly affects the abundance of another node in the network as mediated by competition for finite space. By simulating ecosystems under a diverse range of flow modification scenarios from natural to extreme flooding, drought, and flow homogenization (Fig. 2; see Methods), this work uses network theory to identify alternative potential futures where climate- and human-induced changes lead to significant shifts in the structure of ecological communities.

Results and discussion

Changing flow conditions led to substantial shifts in riparian network properties according to the modelling results, including the number of nodes and links, network connectance, and reciprocity (see Table 1 for definitions). Here, nodes represent the six life-history stages of the five plant guilds, and links represent a significant cross-guild sensitivity (small changes in a vital rate of one node significantly alters abundance of another through a shared dependency on finite space). Therefore, links were not defined a priori, but arose through population dynamics resulting from the models. Increasing the frequency of drought years relative to flood years

led to a simplification of ecological networks (Figs. 2 and 3 and Supplementary Figs. 2 and 3). By contrast, increasing flood year frequency (the frequency of years over the time series that were defined as flood, rather than drought or non-event years) had a less pronounced effect on network completeness and connectance. The greatest simplifications (with significant changepoints, which represent locations in the data where the statistical properties of a sequence of observations change; Table 1) were observed for the number of nodes with increased drought, and the number of links with both increasing drought and flood years (Fig. 3).

Networks were most connected for hydrographs (a record of river discharge over time) similar to the natural flow regime (Table 1), probably due to the fact that species comprising these guilds had evolved under these flow conditions¹¹. Although the number of nodes in networks peaked near the natural flow regime and plateaued with increasing floods, network connectance and reciprocity declined at high flood year frequencies (Fig. 3). High network connectance and reciprocity indicate the existence of compensatory population dynamics, which confer resilience to flood or drought disturbances²¹ and can stabilize communities in the presence of perturbations²². This demonstrates that if one community member experiences a decline in a vital rate or density through external

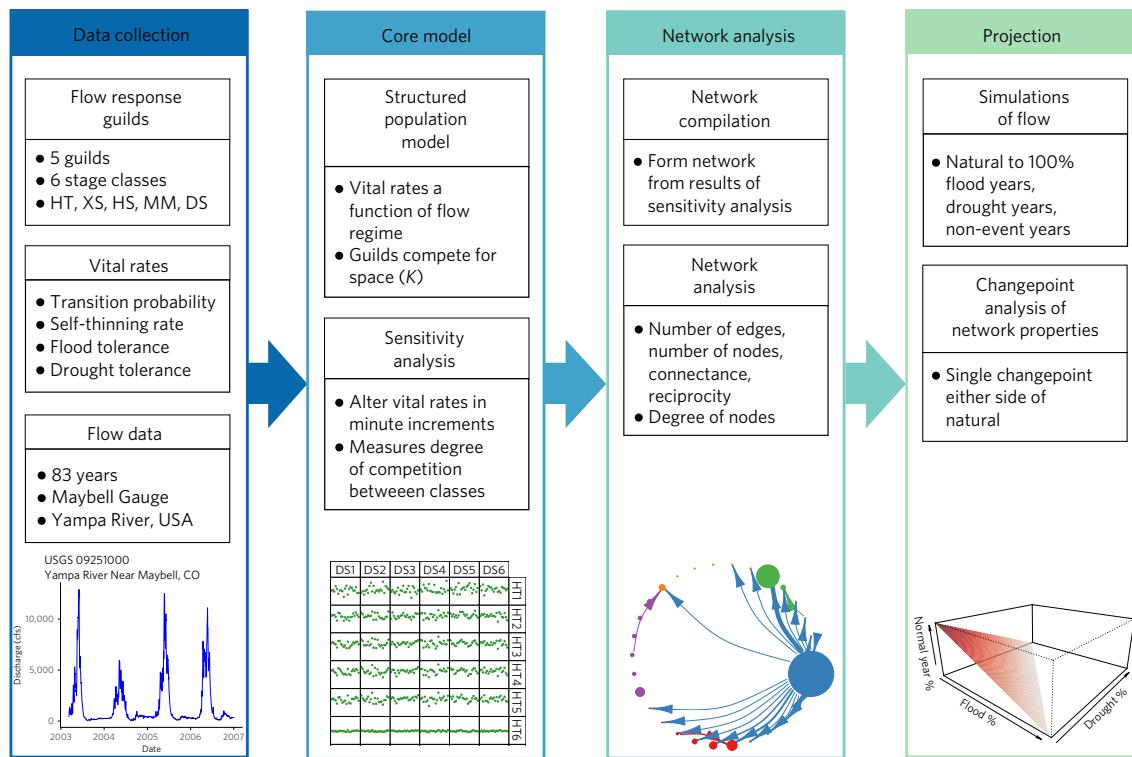


Fig. 1 | Conceptual diagram outlining the workflow used in the analysis, from data collection to projection. The workflow followed four main steps (left to right): data collection (such as the estimation of guild vital rates from field data and experiments, or hydrograph data); model building and analysis (matrix population model, or sensitivity analysis); network analysis; and projection and simulation across a range of altered flow regimes. We used iterated sensitivity analysis to quantify interactions between members of the community and form networks. The effects of flow alteration on networks were explored by projecting ahead 250 years. See Methods for full details. cfs, cubic feet per second.

stressors, others (not experiencing such decline) will respond by taking its place, a beneficial phenomenon for overall biodiversity and ecosystem functioning. Network connectance also influences the stability of communities in ways that affect the long-term persistence of particular community types^{14,23–25}. Connectance plays a fundamental role in the maintenance of biodiversity, with highly connected communities being more robust to species losses in food webs¹⁵ and more resistant to invasion by non-native species²⁶. Losses of interactions can also be precursors to future biodiversity loss²⁷. Therefore, the simplification of networks, particularly with increasing drought, which is predicted to increase widely over the next century²⁸, may predispose networks to collapse¹². In sum, the highly connected networks that we observed under natural flow regime conditions could represent communities that are evolutionarily optimized to the flow components associated with such natural or historic conditions.

Flow homogenization (stable base-flow and reduced flood events, often the result of dam operations) caused network simplification similar to that observed under drought scenarios, reflecting the central role of floods in dryland rivers (Fig. 2 and Supplementary Figs. 4–6). Flow homogenization considerably influences the resident biota of rivers²⁹, and can directly affect the riparian community through reduced water availability, changes in the timing of availability, and lack of flood scouring necessary for seedling recruitment³⁰. Preserving or restoring key components of the natural flow regime, such as flooding, should therefore be a high priority for river managers. Our results and mechanistic modelling approach, which can be tailored for target species, complements an existing literature on experimental flows³¹. Together, these provide the tools required for managers to design prescribed flow releases to maximize the benefits for target species or

overall emergent community properties, such as our metric of network connectance.

The natural flow regime has been severely modified in many river systems through human activities such as dam construction and management for hydropower and water storage and diversion, but components of the flow regime are expected to shift under even the most conservative of climate change scenarios^{7,32}. The results of our drought and homogenization scenarios indicate how influential these environmental changes can be on the organization of river-dependent communities. By incorporating riparian plant guilds that occur in dryland river systems worldwide^{33,34}, these results apply beyond our study system. The particular relevance of the results to dryland regions explains why scenarios involving elevated flood frequency were less problematic than the outcomes involving reduced flooding. Although increasing flood frequency is just one of many predicted changes for the study region, our results help to demonstrate the importance of preserving floods in dynamic river systems. In regions that are less water-scarce, increased flood frequency and magnitude (disturbance rather than water availability) may cause more dramatic shifts in riparian ecosystems^{35,36}. Thus, the widespread nature of flow-altering dams⁴, the pervasive threat of future damming worldwide³, and the adaptation of many riverine organisms to specific attributes of the natural flow regime¹¹, highlight the concerning nature of the demonstrated network-wide effects of flow modification on these individual species, communities and riparian ecosystems overall.

The role of individual nodes within networks revealed guild-specific responses to altered flow conditions. By examining how guild interactions (links in the network) changed in response to hydrology, we identified three distinct node types based on their degree (number of links per node) within the network. Rather than

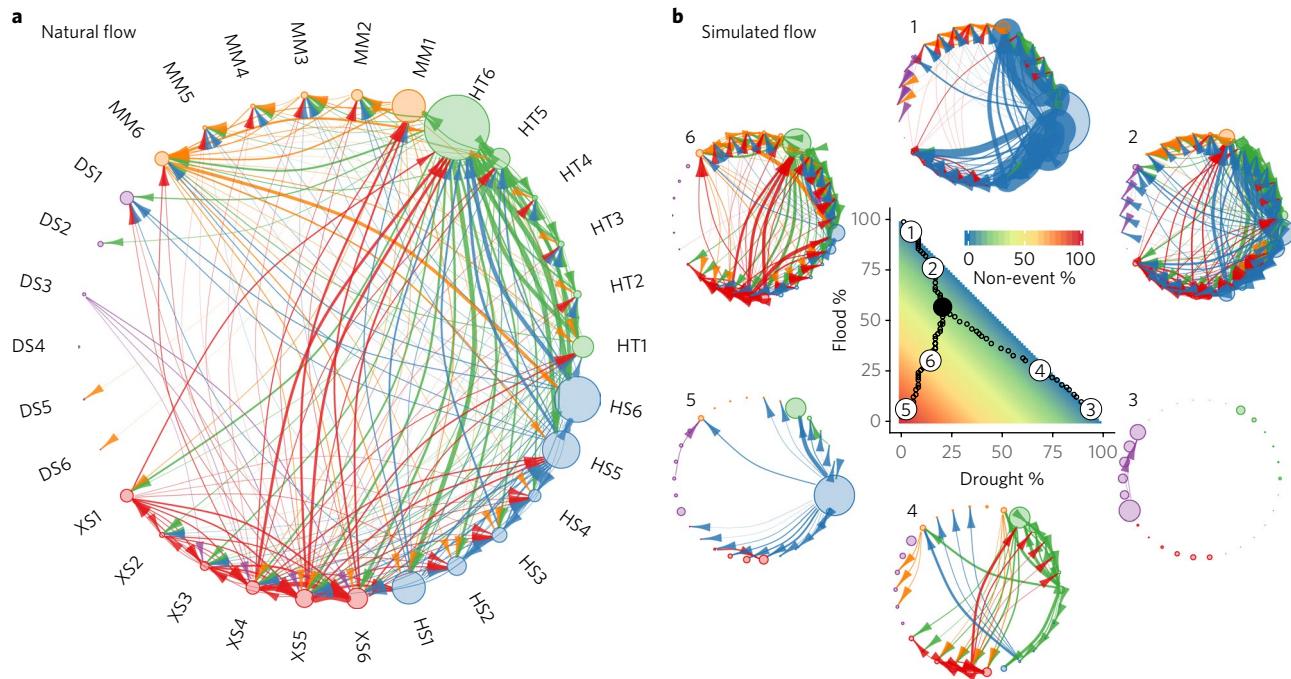


Fig. 2 | Examination of riparian plant networks across a spectrum of possible flow regime futures. **a**, The network simulated according to the natural flow regime. Nodes on the outside of the circle represent the 30 members of the network (5 guilds, 6 stage classes; colour-coded by guild; size scales with abundance) and links represent significant interactions (see Table 1; width = relationship strength; colour matches source node; links are directional). Node order is consistent across networks and all nodes are shown, even if unconnected. The labels on the nodes represent the guild and its stage class (HT, hydroriparian pioneer trees; XS, xeroriparian pioneer shrubs; HS, hydroriparian pioneer shrubs; MM, mesoriparian meadow species; DS, desert shrubs). **b**, Examples of networks at a range of altered flows. Scenarios 1–6 represent the position of networks in this flow-space graph (for example, '1' is an extreme flood scenario and '3' is extreme drought). Three year types are possible in the model: flood, drought and non-event (flow homogenization), and we ran scenarios from the natural flow regime (56.6% flood years) to the extremes of these year types, as shown in the flow-space plot. All simulated scenarios (open circles on the flow-space) are shown in relation to natural flow regime (filled circle). See the Supplementary Information for the complete range of networks (directional and bidirectional) and see Methods for details.

defining interactions a priori, guild interactions emerged from the model via sensitivity analysis, as a direct result of a shared dependency on finite space. Keystone nodes^{18,27,37} were those that had large effects on the overall network, shown by substantial numbers of links outbound to other nodes (Table 1, Fig. 4 and Supplementary Fig. 7). Mutual nodes played a key reciprocal role in the network, by harbouring many bidirectional links, akin to compensatory dynamics²¹. By contrast, passive nodes were those that responded to, rather than influenced, other nodes in the network through receiving many inbound links (Table 1). Keystone nodes were most prevalent in reproductive guild stages, and non-reproductive stages tended to function as passive nodes, having little influence on other stages or guilds.

Adult hydroriparian pioneer trees and xeroriparian pioneer shrubs functioned as keystone nodes at or near the natural flow regime, and declined with both increasing and decreasing flood frequency. Hydroriparian pioneer shrubs, on the other hand, are the dominant riparian guild in many temperate and mesic regions globally^{38–40}, and became keystone nodes at high flood frequency scenarios (Fig. 4). Thus, the model identified critical hydrologic thresholds where alteration to river hydrology caused a change in the identity of the keystone riparian guild. Hydroriparian trees (such as *Populus* spp., *Alnus* spp. and *Eucalyptus* spp.) and hydroriparian shrubs (such as *Salix* spp.) are critical components of riparian plant communities and riparian habitats worldwide. These flood-adapted, phreatophytic woody species provide ecosystem services such as habitat for riparian wildlife, flood mitigation and bank stabilization, modification to microclimate conditions by cooling and increasing humidity, and nutrient cycling³⁹. In riparian

ecosystems globally, there are woody species, both trees and shrubs, that perform keystone roles in riparian communities, including black poplar (*Populus nigra*) in Europe, river red gum (*Eucalyptus camaldulensis*) in Australia, and cottonwood species (*Populus* spp.) in North America^{41–43}. With the loss of native hydroriparian shrubs and trees due to changes in flow regimes, there are often shifts to more xeric and upland species and decreases in riparian habitat quality and complexity^{33,44,45}. These effects, however, depend largely on the baseline hydrologic setting of a river⁴⁶.

The model was sensitive to scenarios where small changes to the flow regime led to substantial reorganization of networks and shifts in the stage-specific ecological role of guilds. Network connectance has been shown to be generally well conserved and insensitive to changing environmental conditions in ecological networks⁴⁷. However, we observed changes in network connectance across the spectrum of flow regime scenarios. Under the increased-drought scenario, relatively small increases in drought frequency led to increases in the number of outbound links for xeroriparian pioneer shrubs, particularly for reproductive stages (stages 4–6; Fig. 4). However, the overall relative space occupied by all stage classes increased similarly for xeroriparian pioneer shrubs (20.7 to 24.1% of K; 95% confidence intervals (CIs) (17.1, 24.3) and (20.9, 27.4), respectively) and hydroriparian pioneer trees (33.4 to 36.7% of K; 95% CIs (29.8, 36.9) and (33.5, 39.8), respectively), with a small shift to drier conditions from the natural flow regime (56.6% to 45.7% flood years). Increasing drought frequencies can lead to loss of the unique ecosystem structure and services that distinguish riparian ecosystems from surrounding uplands. Due to available space opening up in increasingly flood-free conditions, the degree of

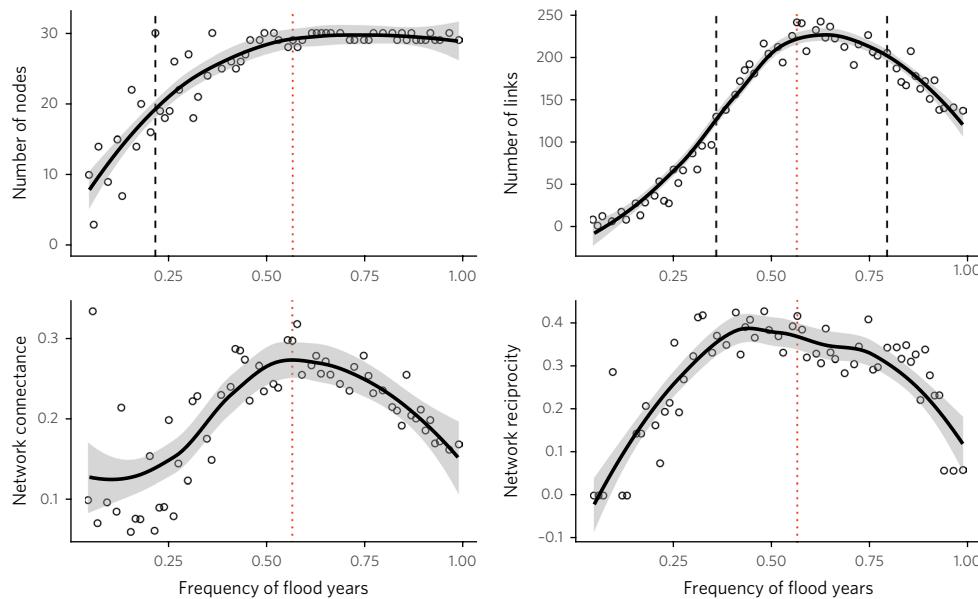


Fig. 3 | Summary metrics of full ecological networks spanning the spectrum of flow extremes from extreme drought to flood. See Table 1 for definitions of terms. Network metrics were calculated only on nodes that were connected; that is, isolated nodes in Fig. 2 were not incorporated into networks, but unconnected does not mean extinct. Trend lines represent locally weighted smoothing (LOESS) fits, accompanied by 95% confidence intervals (shaded areas). The natural flow regime is shown as the dotted line. Dashed lines represent significant changepoints in the series. We limited the search for changepoints to a maximum of one per scenario (that is, natural flow regime to each of the three extreme flows). Each point represents an approximately 1.5% change in the frequency of flood and drought years. ‘Flood years’ are years when the peak discharge exceeds $280 \text{ m}^3 \text{ s}^{-1}$. See the Supplementary Information for homogenization scenario results.

each node was lower than more natural or flood-dominated conditions, which was true even for the drought specialist desert shrub. Increased flood frequency, however, shifted the community towards guilds more tolerant of wet conditions, with stages five and six of flood-tolerant hydriparian pioneer shrubs being keystone nodes in flood-dominated conditions. The overall space occupied by all hydriparian pioneer shrub stages reflected this dominance when moving from natural flow conditions to slightly wetter conditions (63.8% flood years; 43.1 to 51.0% of K ; 95% CIs (34.8, 51.4) and (43.6, 58.4), respectively). By contrast, the desert shrub guild played little role in any network other than being a passive responder, dominating only under the most severe drought conditions in response to the decline of other river-dependent guilds⁴⁸. Such terrestrialization has been demonstrated in response to groundwater depletion linked to streamflow alteration in a number of studies^{48,49}.

Conclusions

Network connectance declined with increasing flow alteration. River-dependent communities have evolved over millennia and have been tailored by natural selection to the volume and seasonal variability in natural flow regimes¹¹. Examining how ecological networks change across a spectrum of environmental conditions, we show that moving even modestly away from this natural flow regime can have detrimental effects on the organization of ecological networks, and conversely, how minor adjustments in managed flow regimes may restore necessary flow components for riparian ecosystem functioning (Fig. 3). Highly connected networks represent communities that are optimized at these conditions, having evolved mechanisms to enable persistence in the face of a wide variety of perturbations including and beyond flow regime dynamics. Connectance has implications for community stability^{14,23–25}, robustness^{12,15} and resistance to invasion²⁶; and here indicates the presence of compensatory dynamics stabilizing aggregate community properties²². Being able to explore and visualize the consequences of a changing environment on the structure and emergent properties of

highly complex ecological communities, and identify and predict the specific environmental conditions that enable a particular guild to perform a keystone role in the community, highlights the benefits of the mechanistic and network-based approach employed in the present study.

Keystone status is affected by changes to the natural flow regime. Removing floods, in particular, led to a loss of keystone status of hydriparian pioneer trees. Loss of keystone guilds leads to changes in fundamentally important ecosystem services, such as habitat provision for riparian wildlife, flood mitigation and bank stability, microclimatic regulation, and nutrient cycling³⁹. Conversely, under scenarios of increased flooding, the keystone status of hydriparian pioneer tree was replaced by hydriparian pioneer shrub. Because these two guilds have different edaphic requirements and ecological roles, it is important to predict which guilds will function in a keystone role under future flow regime scenarios. Thus, our network analysis facilitates proactive management for species and life stages of interest under future scenarios.

Floods are fundamental to the maintenance of complex ecological networks. Floods, despite their negative effects on human infrastructure, are associated with many beneficial and necessary processes that enhance the diversity of riverine systems and the robustness and resilience of ecological networks. Although much focus has been on drought, flow homogenization (due to damming) may be equally detrimental to riparian communities. Therefore, managers should strive to preserve or restore natural flow dynamics, particularly floods, as a vital driver of the ecology of rivers³¹. Through connecting flood and drought distributions directly to complex multi-guild networks, our modelling approach highlights the consequences of future climates on riparian networks. Such a mechanistic modelling approach allows us to forecast the nonstationary effects that climate change is expected to impose on future river flow regimes, and equips managers seeking to mitigate these impacts by enabling detailed projections of potential flow futures.

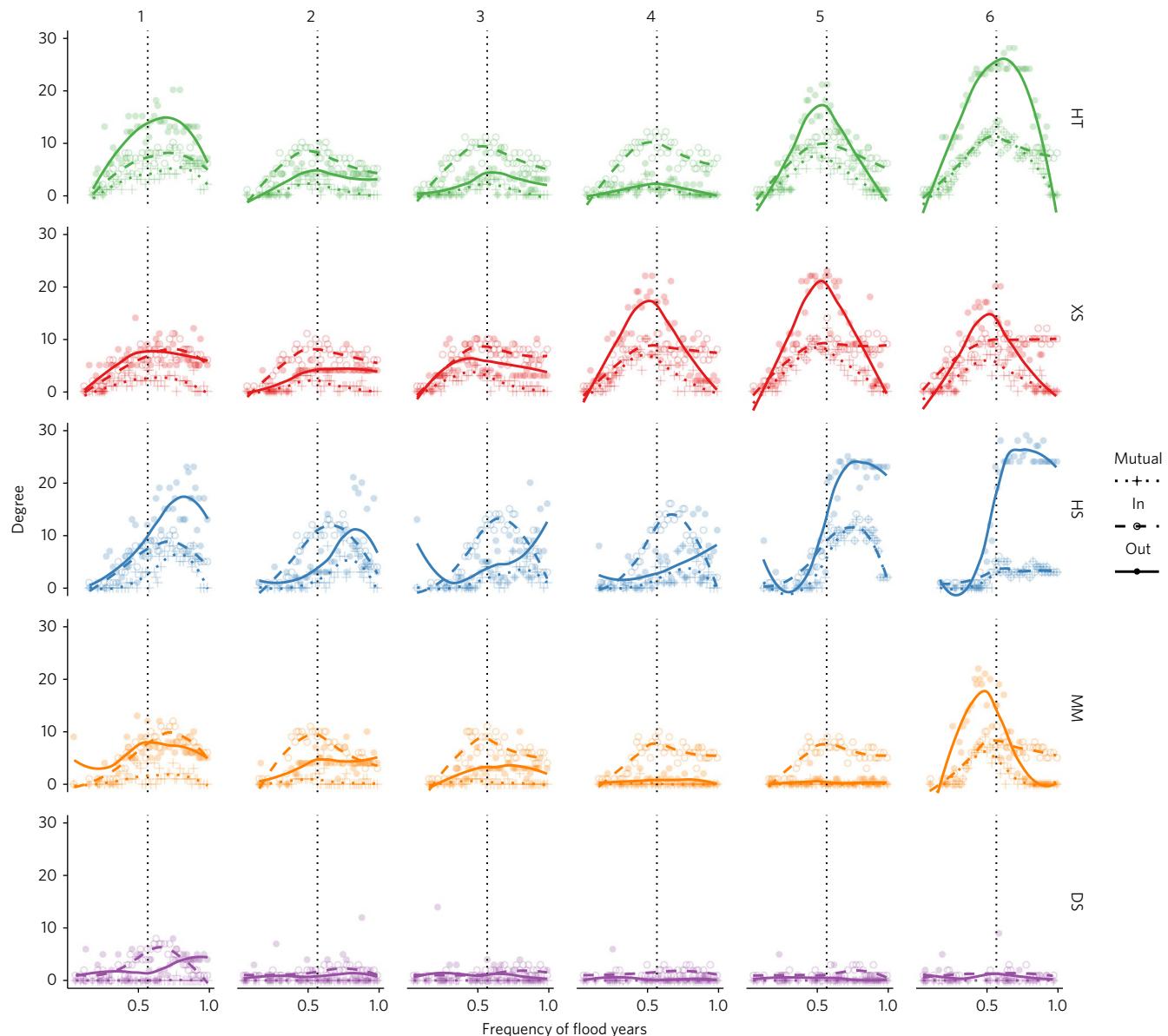


Fig. 4 | Degree (number of links connected to the node) of each network node in relation to flow state. All five guilds (as rows), each with six stage classes (as columns 1–6) are shown. Scenarios were run from 100% drought years to 100% flood years (through the natural flow regime, indicated as the dotted line). Inward, outward and mutual relationships are shown. Trend lines represent LOESS fits. Each point represents an approximately 1.5% change in the frequency of flood and drought years. ‘Flood years’ are years when the peak discharge exceeds $280 \text{ m}^3 \text{s}^{-1}$. See the Supplementary Information for homogenization scenario results.

Methods

Study guilds. We explored population dynamics in five riparian plant guilds that represent riparian assemblages across a wide variety of locations: hydroriparian pioneer tree (HT), xeroriparian pioneer shrub (XS), hydroriparian pioneer shrub (HS), mesoriparian meadow species (MM), and desert shrub (DS) (Table 1). These guilds represent flow-response guilds¹⁹: groups of plants with similar morphological and physiological traits and therefore responses to water availability and fluvial disturbance.

Experimental design and model structure. We employed a stage-structured coupled stochastic population model to explore scenarios of the flow regime spanning extreme drought to extreme flood frequencies, as well as flow homogenization (Fig. 2). Our approach followed a general modelling framework published previously¹⁰. The model allows guilds of species that share similar vital rates to simultaneously occupy a dynamic floodplain zone. A guild j is described by a stage-based matrix $N_j(t+1) = A_j(t)N_j(t)$, where $N_j(t)$ is a vector containing stage abundances and $A_j(t)$ is a set of transition matrices that fluctuate according to variation in the hydrograph. Population dynamics of each guild are determined primarily by how their vital rate parameters (mortality, fecundity and self-

thinning) are affected by flooding and drought. Detailed definitions of vital rate parameters for a subset of the guilds have been published previously⁵⁰. All analyses were performed in R 3.2.3⁵¹.

Each of our study guilds comprised six stage classes from seedling to reproductive adult¹⁰, and are hereafter referred to as network nodes (vertices; $n = 30$). We obtained vital rates for the five guilds from a variety of sources, including field studies and historical aerial photographs^{10,50}. While these vital rates were gathered for a specific site and suite of species, the guild approach enables the transferability of modelling approaches between systems and regions.

The model operates at the reach scale, with total potential floodplain habitat, K , representing the maximum space available for occupation. Floods, droughts, and senescence can reduce the population size of guilds through interacting with their vital rates, in turn opening space for recruitment in the next cycle. The model keeps K constant across years, but changing river dynamics are accounted for broadly through the spatially implicit nature of the model⁵⁰. That is, the spatially implicit structure allows examining population dynamics without needing an understanding of the spatial dynamics of vegetation or river geomorphology^{10,50}.

We used 83 years of hydrograph data from the undammed Yampa River near Maybell, Colorado, USA (US Geological Survey gage number 09251000). Based on

river geomorphology, years when peak discharge exceeded $280 \text{ m}^3 \text{s}^{-1}$ were flood years, and years in which floods remained below $210 \text{ m}^3 \text{s}^{-1}$ were drought years. Years falling in between were considered non-event years. Matrix projection was accomplished in annual time steps. To project the model forward in time, a year was drawn at random from the time series and the corresponding flow metrics were calculated for that year (flood decline rate, timing, and whether it was a flood, drought or non-event year). In flood or drought years, all guilds were subject to guild-specific flood or drought mortality, respectively, in addition to baseline transition probabilities. Given the strong role that predictability of temporal environmental fluctuations can have on biodiversity⁵², an interesting phenomenon to consider in future expansions of this approach is the role of temporal clustering of river flow regimes that incorporate supra-annual climatic cycles.

Sensitivities and network analysis. We used model iterated sensitivity analysis to quantify how small changes in a vital rate (flood susceptibility vital rate, in this case) of one node affects all other nodes within the community. We used iterated sensitivity analysis because it is not possible to obtain an analytical sensitivity from a stochastic, coupled structured population model except under limited circumstances. Iterated sensitivity analysis approximates taking the partial derivative of a population variate with respect to the parameter of interest. This approach differs from those related to the 'Community' matrix (or other Jacobian matrices), where interaction strengths represent functional relationships underlying species interactions, and 'Net Effects' matrices (negative inverse of the Jacobian matrix) in turn enable quantitative predictions of effects of perturbations to one species on all others in the matrix⁵³.

We changed a given vital rate in increments of 0.001 on either side of its actual value for a total of 20 increments. The model was then projected 250 years into the future for each iterated step, and population sizes for each node were averaged for the final 100 years (150–250) and recorded. Each of these steps was repeated 1,000 times to estimate the repeatability of a given effect. The entire process was repeated for each of the flow alteration scenarios (Fig. 1). We calculated sensitivity as the slope of the regression line between the given vital rate value (20 incremental steps) and the average node population size. Sensitivities were calculated for all pairwise node combinations within the network. This allowed the calculation of standard sensitivities (the response of a guild to a change in one of its own vital rates) as well as cross-guild sensitivities (the response of one guild to a change in a vital rate of another guild, as mediated by competition for available space). For example, changes in abundance of a guild, as a result of increased susceptibility to flooding, can have a cascading effect through the ecosystem by opening space for another guild to establish after a recruitment event. Considering this is a hydrograph-driven model and the fact that these riparian plants are inherently linked to floods, the choice of the flood susceptibility vital rate was clear, but other vital rates could also be used to form network links when examining other systems or particular management objectives.

Cross-guild sensitivities were used to create ecological networks, where we quantified the effects of changes to each node's vital rate on abundances of all others in the river reach (also termed sensitivity networks¹⁰). A link (edge) was created between two nodes only where a clear linear relationship was present. These interactions emerge through indirect competition for space on the landscape as each guild occupies a portion of K , the total amount of riparian habitat available to all guilds in a given riparian reach. Occupying space makes it unavailable for new recruitment for another guild, thereby affecting each other's population dynamics. We used a cutoff of $R^2 \geq 0.30$ and $P < 0.01$ for sensitivities to be included as links. Thus, while a species may still be present in a community, it was not included as a node in a network if it had no interactions meeting this criterion. For specific relationships discussed in the text, we also calculated 95% confidence intervals on the simulated data to determine differences in abundances of particular guilds under differing flow regimes. We compiled networks using the 'graph.data.frame' function in the igraph R package⁵⁴. An example of the raw cross-guild regression relationships (at the natural flow regime) that were used to compile networks is given in Supplementary Fig. 1.

We analysed network properties by calculating the number of nodes and links, their connectance (the ratio of the number of links to the maximum possible number of links), and reciprocity (the proportion of mutual links; where there is a two-way relationship). Depending on the scenario, stage classes of particular guilds may have been present in very low abundances during the final 100-year data collection period, and potentially only briefly. Thus, while we included all nodes in the graphs for visualization, we calculated metrics on networks with unconnected nodes (where there were no sensitivity relationships that met our criteria) removed. That is, we considered non-linked nodes as non-members for this purpose. Therefore, missing nodes are those that are no longer linked with any other nodes in the network, based on our criteria of a significant interaction, and node number can drop below 30, despite lack of local extinctions. We also examined the degree (number of links connected to the node, or how well connected that node is in the network) of each guild stage class in the model. We calculated links out from a node and into a node, as well as bidirectional links.

Networks were compiled for the full suite of flow scenarios spanning from the natural flow regime to 100% drought years, 100% flood years and 100% non-event years. Thus, the entire process of model runs, network building and network analysis described above was repeated at each flow regime scenario (Fig. 1). Vital

rates were held constant across all flow regime scenarios (our goal was to simulate ecological rather than evolutionary dynamics), but hydrograph parameters were allowed to vary across an array of flow regime scenarios. For this analysis, we explored the sensitivity of networks to changes in one vital rate at time; we did not explore the possibility of multiple vital rates changing simultaneously. To simulate increased flood (or drought) frequency, we successively changed 2 of the 83 gauge years so that eventually all of them became flood (or drought) years (42 iterations in total for the 100% drought to flood scenario). To simulate flow homogenization due to damming, we successively changed year types, starting at natural flow regime frequency, until all of them became non-event years. Although our simulations were limited to modification of the frequency of floods and droughts, the core matrix model incorporates and varies other aspects of the natural flow regime depending on the year drawn from the flow vector (duration, timing, and interannual variability; magnitude is fixed as a threshold function). Thus, it is also possible to examine the network-wide effects of shifts in the timing and duration of floods using this approach.

To examine whether thresholds existed in network properties with altered flow scenarios, we tested for changepoints in the data. These represent precise locations in the ordered data series where the statistical properties of a sequence of observations change. We searched for single changepoints in the mean ('cpt.mean' function) moving from the natural flow regime through to extreme flood, drought and non-event scenarios using the changepoint R package⁵⁵. To do this, we used the 'at most one change' (AMOC) method and the MBIC (modified bayesian information criterion) penalty.

Life Sciences Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

Code availability. The core matrix model in R is available on figshare (<http://dx.doi.org/10.6084/m9.figshare.4652608>). Sensitivities can be calculated from this script using an iterative outer loop as detailed in the Methods.

Data availability. All data required to run the model, including the flow hydrograph from the Yampa River and guild vital rates, is available on figshare (<http://dx.doi.org/10.6084/m9.figshare.4652608>).

Received: 4 March 2017; Accepted: 11 October 2017;

Published online: 27 November 2017

References

1. Poff, N. L. et al. The natural flow regime. *Bioscience* **47**, 769–784 (1997).
2. Nilsson, C., Reidy, C. A., Dynesius, M. & Revenga, C. Fragmentation and flow regulation of the world's large river systems. *Science* **308**, 405–408 (2005).
3. Zarfl, C., Lumsdon, A. E., Berlekamp, J., Tydecks, L. & Tockner, K. A global boom in hydropower dam construction. *Aquat. Sci.* **77**, 161–170 (2014).
4. Poff, N. L., Olden, J. D. J., Merritt, D. M. & Pepin, D. M. Homogenization of regional river dynamics by dams and global biodiversity implications. *Proc. Natl Acad. Sci. USA* **104**, 5732–5737 (2007).
5. Flanagan, N. E., Richardson, C. J. & Ho, M. Connecting differential responses of native and invasive riparian plants to climate change and environmental alteration. *Ecol. Appl.* **25**, 753–767 (2015).
6. Naiman, R. J. & Déamps, H. The ecology of interfaces: riparian zones. *Annu. Rev. Ecol. Syst.* **28**, 621–658 (1997).
7. Reynolds, L. V., Shafrroth, P. B. & Poff, N. L. Modeled intermittency risk for small streams in the Upper Colorado River Basin under climate change. *J. Hydrol.* **523**, 768–780 (2015).
8. Lu, X. et al. Drought rewrites the cores of food webs. *Nat. Clim. Change* **6**, 875–878 (2016).
9. Ledger, M. E., Brown, L. E., Edwards, F. K., Milner, A. M. & Woodward, G. Drought alters the structure and functioning of complex food webs. *Nat. Clim. Change* **3**, 223–227 (2012).
10. Lytle, D. A., Merritt, D. M., Tonkin, J. D., Olden, J. D. & Reynolds, L. V. Linking river flow regimes to riparian plant guilds: a community-wide modeling approach. *Ecol. Appl.* **27**, 1338–1350 (2017).
11. Lytle, D. A. & Poff, N. L. Adaptation to natural flow regimes. *Trends Ecol. Evol.* **19**, 94–100 (2004).
12. Strona, G. & Lafferty, K. D. Environmental change makes robust ecological networks fragile. *Nat. Commun.* **7**, 1–7 (2016).
13. Ings, T. C. et al. Ecological networks — beyond food webs. *J. Anim. Ecol.* **78**, 253–269 (2009).
14. Thébaud, E. & Fontaine, C. Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science* **329**, 853–856 (2010).
15. Dunne, J. A., Williams, R. J. & Martinez, N. D. Network structure and biodiversity loss in food webs: robustness increases with connectance. *Ecol. Lett.* **5**, 558–567 (2002).
16. Poisot, T., Stouffer, D. B. & Gravel, D. Beyond species: why ecological interaction networks vary through space and time. *Oikos* **124**, 243–251 (2015).

17. Thompson, P. L. & Gonzalez, A. Dispersal governs the reorganization of ecological networks under environmental change. *Nat. Ecol. Evol.* **1**, 0162 (2017).
18. Stouffer, D. B., Sales-Pardo, M., Sirer, M. I. & Bascompte, J. Evolutionary conservation of species' roles in food webs. *Science* **335**, 1489–1492 (2012).
19. Merritt, D. M., Scott, M. L., Poff, N. L., Auble, G. T. & Lytle, D. A. Theory, methods and tools for determining environmental flows for riparian vegetation: riparian vegetation-flow response guilds. *Freshwater Biol.* **55**, 206–225 (2010).
20. Milly, P. C. D., Dunne, K. A. & Vecchia, A. V. Global pattern of trends in streamflow and water availability in a changing climate. *Nature* **438**, 347–350 (2005).
21. Gonzalez, A. & Loreau, M. The causes and consequences of compensatory dynamics in ecological communities. *Annu. Rev. Ecol. Evol. Syst.* **40**, 393–414 (2009).
22. Brown, B. L., Downing, A. L. & Leibold, M. A. Compensatory dynamics stabilize aggregate community properties in response to multiple types of perturbations. *Ecology* **97**, 2021–2033 (2016).
23. Allesina, S. & Tang, S. Stability criteria for complex ecosystems. *Nature* **483**, 205–208 (2012).
24. Mougi, A. et al. Diversity of interaction types and ecological community stability. *Science* **337**, 349–351 (2012).
25. May, R. M. Will a large complex system be stable? *Nature* **238**, 413–414 (1972).
26. Baiser, B., Russell, G. J. & Lockwood, J. L. Connectance determines invasion success via trophic interactions in model food webs. *Oikos* **119**, 1970–1976 (2010).
27. Aizen, M. A., Sabatino, M. & Tylianakis, J. M. Specialization and rarity predict nonrandom loss of interactions from mutualist networks. *Science* **335**, 1486–1489 (2012).
28. Dai, A. Drought under global warming: a review. *Rev. Clim. Change* **2**, 45–65 (2011).
29. Bunn, S. E. & Arthington, A. H. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environ. Manage.* **30**, 492–507 (2002).
30. Rood, S. B., Goater, L. A., Mahoney, J. M., Pearce, C. M. & Smith, D. G. Floods, fire, and ice: disturbance ecology of riparian cottonwoods. *Can. J. Bot.* **85**, 1019–1032 (2007).
31. Olden, J. D. et al. Are large-scale flow experiments informing the science and management of freshwater ecosystems? *Front. Ecol. Environ.* **12**, 176–185 (2014).
32. Rood, S. B. et al. Declining summer flows of Rocky Mountain rivers: changing seasonal hydrology and probable impacts on floodplain forests. *J. Hydrol.* **349**, 397–410 (2008).
33. Stromberg, J. C. & Merritt, D. M. Riparian plant guilds of ephemeral, intermittent and perennial rivers. *Freshwater Biol.* **61**, 1259–1275 (2016).
34. Aguiar, F. C., Cerdeira, J. O., Martins, M. J. & Ferreira, M. T. Riparian forests of southwest Europe: are functional trait and species composition assemblages constrained by environment? *J. Veg. Sci.* **24**, 628–638 (2013).
35. Gurnell, A. M., Bertoldi, W. & Corenblit, D. Changing river channels: the roles of hydrological processes, plants and pioneer fluvial landforms in humid temperate, mixed load, gravel bed rivers. *Earth Sci. Rev.* **111**, 129–141 (2012).
36. Hupp, C. Hydrology, geomorphology and vegetation of coastal plain rivers in the south-eastern USA. *Hydrol. Process.* **14**, 2991–3010 (2000).
37. Lewinsohn, T. & Cagnolo, L. Keystones in a tangled bank. *Science* **335**, 1449–1451 (2012).
38. Friedman, J. M. et al. Dominance of non-native riparian trees in western USA. *Biol. Invasions* **7**, 747–751 (2005).
39. Kominoski, J. S. et al. Forecasting functional implications of global changes in riparian plant communities. *Front. Ecol. Environ.* **11**, 423–432 (2013).
40. Rivaes, R. P. et al. Modeling the evolution of riparian woodlands facing climate change in three European rivers with contrasting flow regimes. *PLoS ONE* **9**, e110200 (2014).
41. Doody, T. M. et al. Quantifying water requirements of riparian river red gum (*Eucalyptus camaldulensis*) in the Murray-Darling Basin, Australia – implications for the management of environmental flows. *Ecohydrology* **8**, 1471–1487 (2015).
42. Kath, J., Le Brocq, A., Leyer, I. & Mosner, E. Hydrological and land use determinants of *Eucalyptus camaldulensis* occurrence in floodplain wetlands. *Austral Ecol.* **39**, 643–655 (2014).
43. Guilloty-Froget, H., Muller, E., Barsoum, N. & Hughes, F. M. R. Dispersal, germination, and survival of *Populus nigra* L. (Salicaceae) in changing hydrologic conditions. *Wetlands* **22**, 478–488 (2002).
44. Middleton, B. A. & Souter, N. J. Functional integrity of freshwater forested wetlands, hydrologic alteration, and climate change. *Ecosyst. Health Sustain.* **2**, e01200 (2016).
45. Andersen, D. C. & Cooper, D. J. Dams, floodplain land use, and riparian forest conservation in the semiarid Upper Colorado River Basin, USA. *Environ. Manage.* **40**, 453–475 (2007).
46. Belmar, O., Bruno, D., Martínez-Capel, F., Barquín, J. & Velasco, J. Effects of flow regime alteration on fluvial habitats and riparian quality in a semiarid Mediterranean basin. *Ecol. Indic.* **30**, 52–64 (2013).
47. Tylianakis, J. M., Tscharntke, T. & Lewis, O. T. Habitat modification alters the structure of tropical host-parasitoid food webs. *Nature* **445**, 202–205 (2007).
48. Merritt, D. M. & Bateman, H. L. Linking stream flow and groundwater to avian habitat in a desert riparian system. *Ecol. Appl.* **22**, 1973–1988 (2012).
49. Shafron, P. B., Auble, G. T., Stromberg, J. C. & Patten, D. T. Establishment of woody riparian vegetation in relation to annual patterns of streamflow, Bill Williams River, Arizona. *Wetlands* **18**, 577–590 (1998).
50. Lytle, D. A. & Merritt, D. M. Hydrologic regimes and riparian forests: a structured population model for cottonwood. *Ecology* **85**, 2493–2503 (2004).
51. R Core Team *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna, 2015).
52. Tonkin, J. D., Bogan, M. T., Bonada, N., Ríos-Touma, B. & Lytle, D. A. Seasonality and predictability shape temporal species diversity. *Ecology* **98**, 1201–1216 (2017).
53. Novak, M. et al. Characterizing species interactions to understand press perturbations: what is the community matrix? *Annu. Rev. Ecol. Evol. Syst.* **47**, 409–432 (2016).
54. Csardi, G. & Nepusz, T. The igraph software package for complex network research. *InterJournal, Complex Syst.* 1695 (2006).
55. Killick, R. & Eckley, I. A. Changepoint: an R package for changepoint analysis. *J. Stat. Softw.* **58**, 1–19 (2014).

Acknowledgements

We thank N. L. Poff, A. Ruhi and members of the OSU Integrative Biology journal club for comments on earlier versions of the manuscript. Dinosaur National Monument and the US Bureau of Reclamation supported the research that led to the vital rate data. Funding was provided in part by the US Department of Defense (SERDP RC-2511) and the US Department of Agriculture Forest Service.

Author contributions

J.D.T. and D.A.L. designed the study, developed the model and ran analyses; D.A.L. and D.M.M. compiled vital rate information; J.D.T. wrote the first draft of the manuscript in close collaboration with D.A.L., and all authors contributed substantially to writing in subsequent drafts.

Competing interests

The authors declare no competing financial interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41559-017-0379-0>.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to J.D.T.

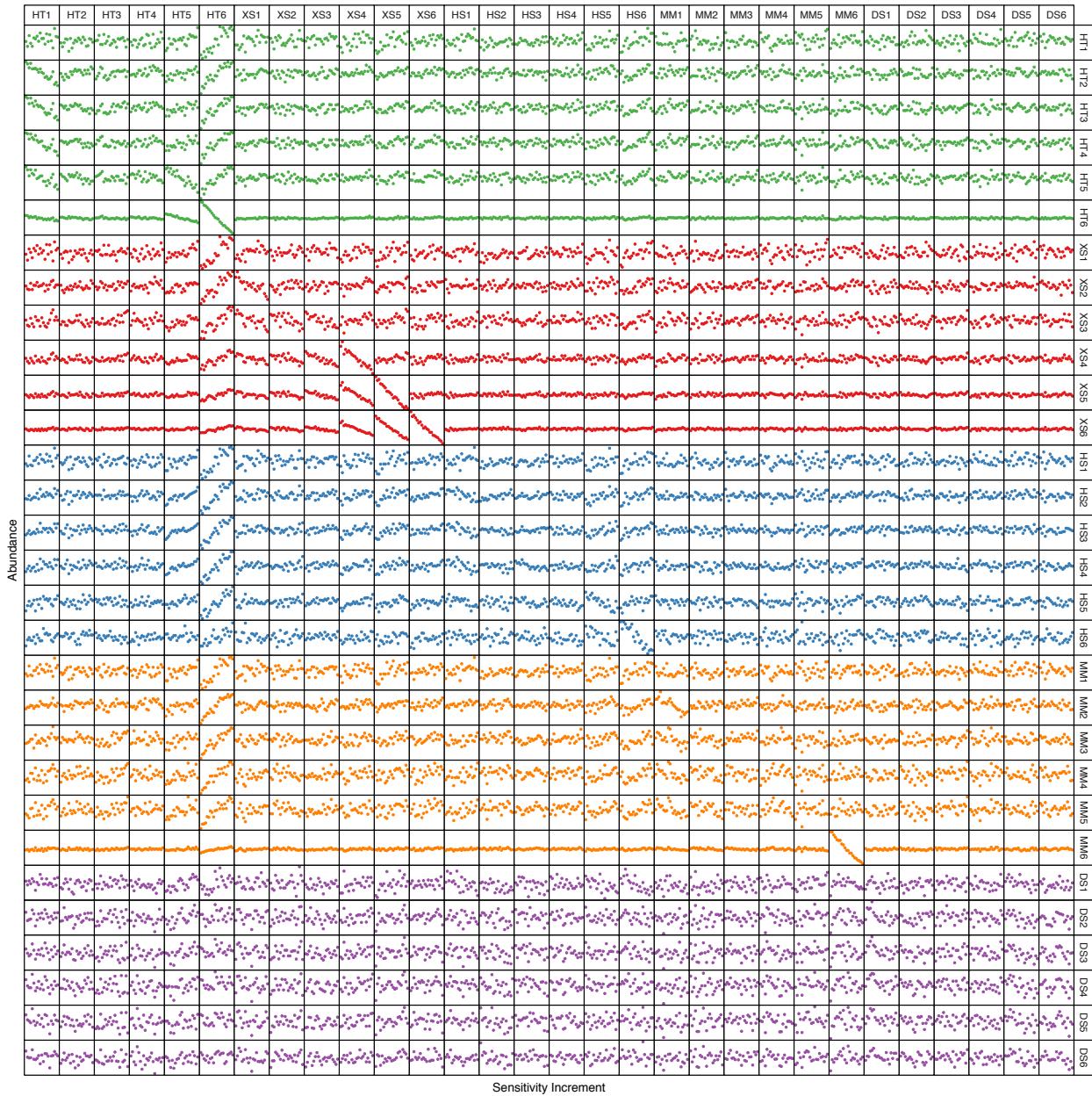
Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

In the format provided by the authors and unedited.

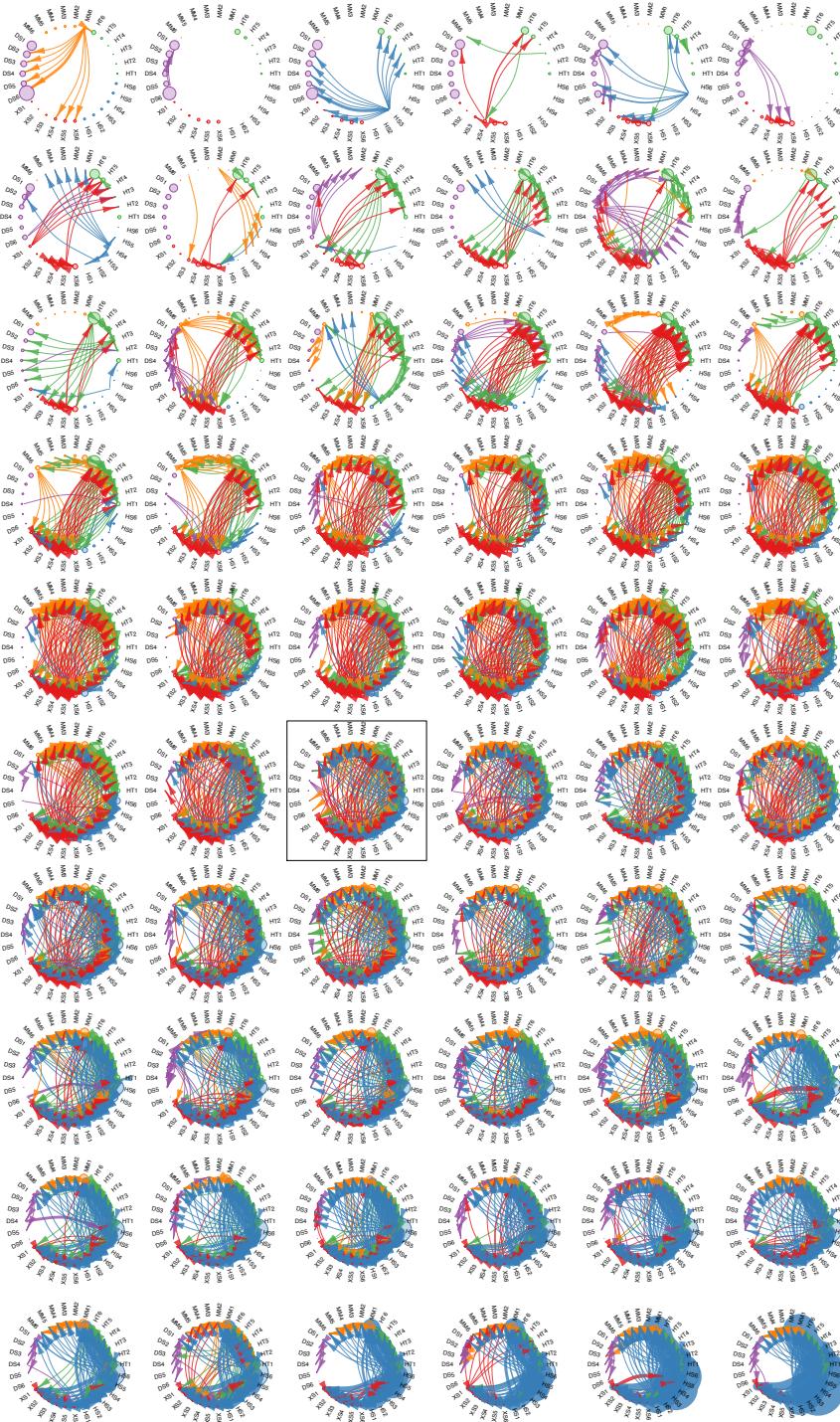
Flow regime alteration degrades ecological networks in riparian ecosystems

Jonathan D. Tonkin^{1*}, David. M. Merritt², Julian D. Olden¹, Lindsay V. Reynolds¹ and David A. Lytle¹

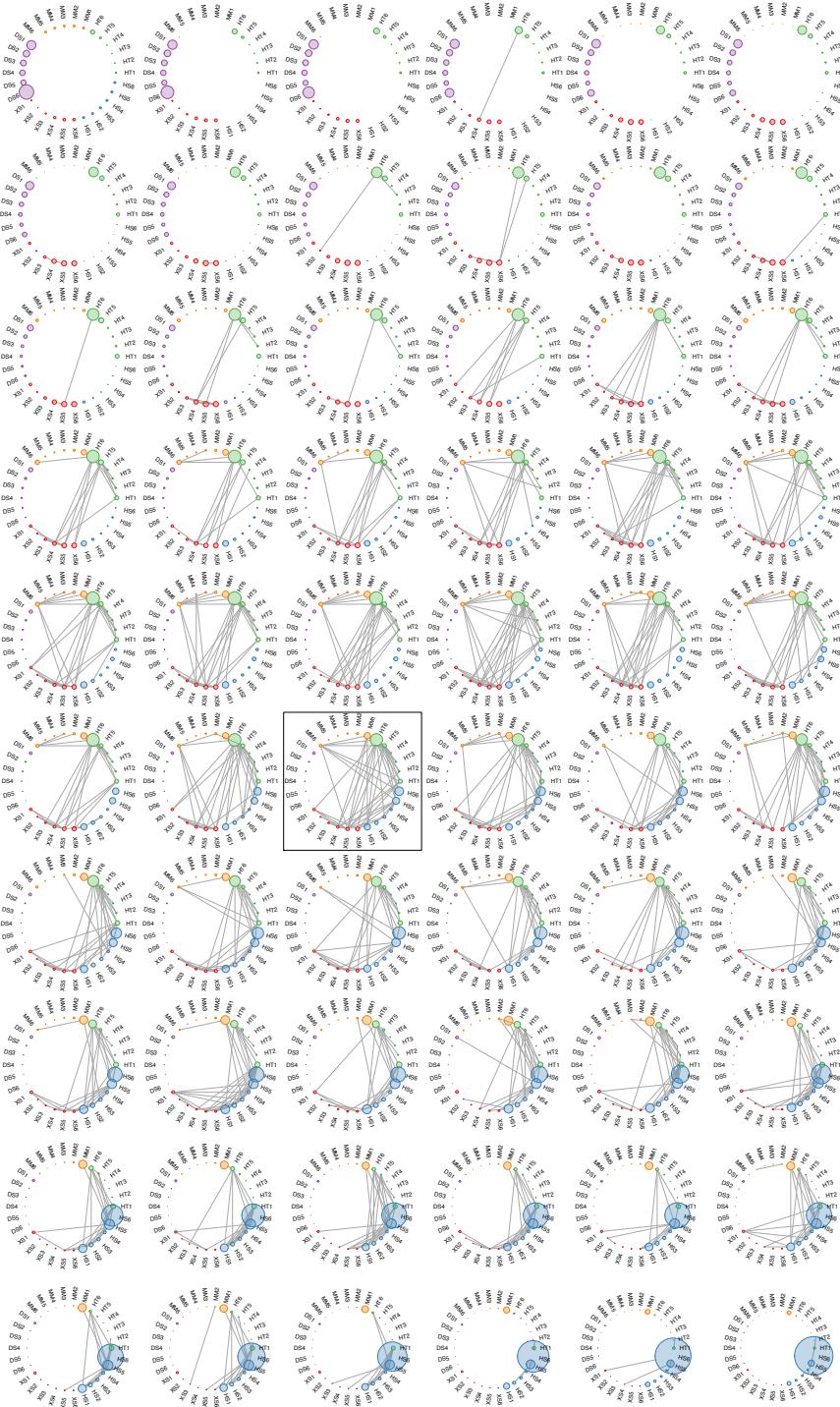
¹Department of Integrative Biology, Oregon State University, Corvallis, OR 97331, USA. ²Watershed, Fisheries, Wildlife, Air and Rare Plants Staff and the National Stream and Aquatic Ecology Center, U.S.D.A. Forest Service, Fort Collins, CO 80526, USA. ³School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98195, USA. *e-mail: jdttonkin@gmail.com



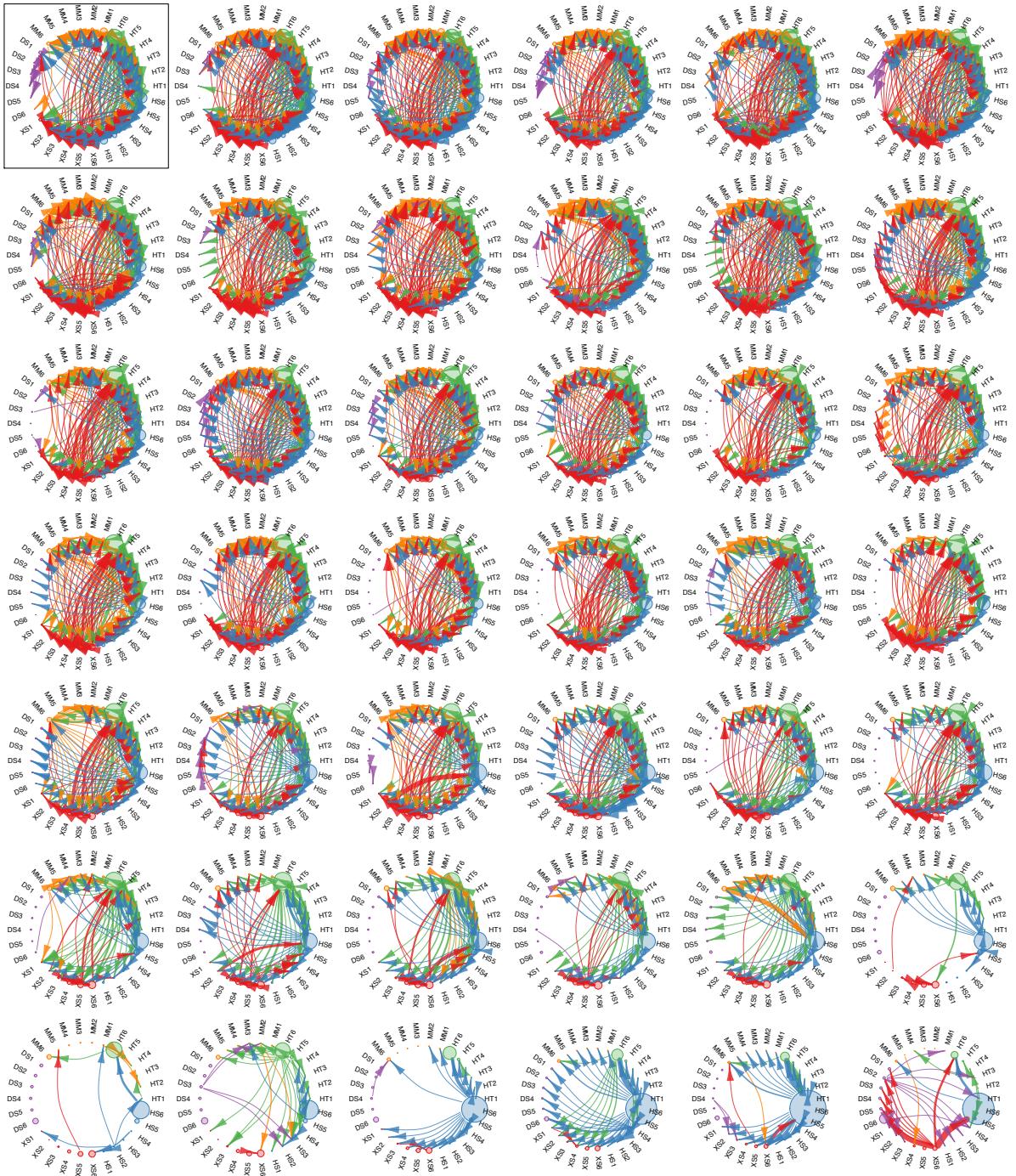
Supplementary Figure 1: Raw sensitivity analysis data at the natural flow regime (56.6% flood years). These plots show the regressions that were used to compile the sensitivity-based ecological network. Each row or column is a stage class within each guild. Columns represent the modified guild stage class and rows represent responses (e.g. the first column shows the responses of all guilds to changes to HT1). Points are color coded by the response guild, not the modified guild. The y-axis represents the abundance of each guild under each setting and the x axis represents the flood tolerance vital rate value resulting from the 20 incremental changes made in the sensitivity analysis, with the middle point being the primary vital rate value. HT: hydroriparian pioneer trees; XS: xeroriparian pioneer shrubs; HS: hydroriparian pioneer shrubs; MM: mesoriparian meadow species; DS: desert shrubs.



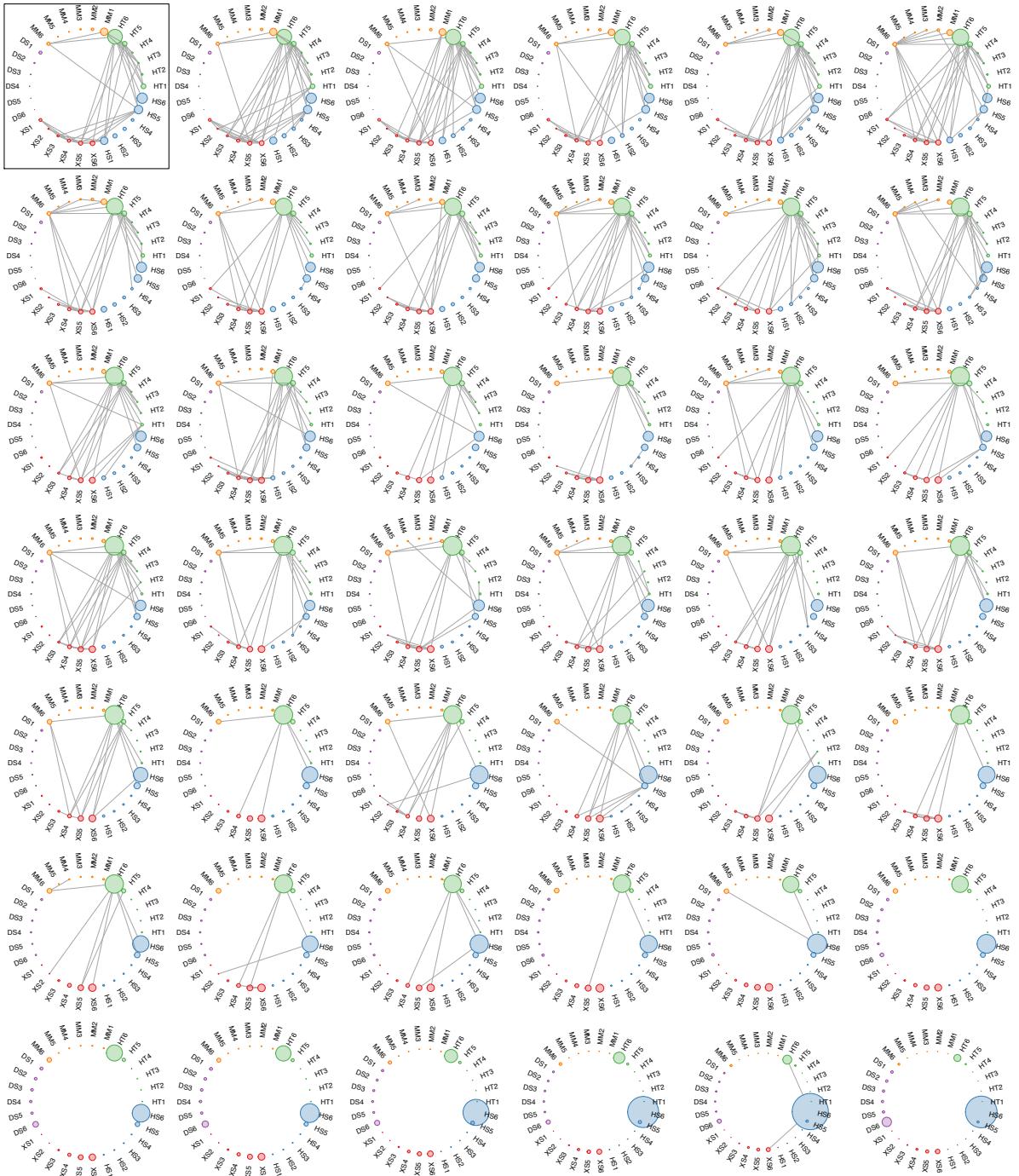
Supplementary Figure 2: Full spectrum of ecological networks ranging from extreme drought to extreme flood scenarios, showing directional links. The natural flow regime (56.6% flood years) is shown in the box. Nodes on the outside of the circle represent the 30 members of the network (5 guilds, 6 stage classes; color coded by guild), and links represent significant interactions (see Table 1; width = relationship strength; color matches source node; links are directional). Node size increases with the abundance of that stage class. Node order is consistent across networks and all nodes are shown, even if unconnected. The labels on the nodes represent the guild and its stage class (1-6). HT: hydroriparian pioneer trees; XS: xeroriparian pioneer shrubs; HS: hydroriparian pioneer shrubs; MM: mesoriparian meadow species; DS: desert shrubs. Each network represents an approximately 1.5% change in the frequency of flood and drought years.



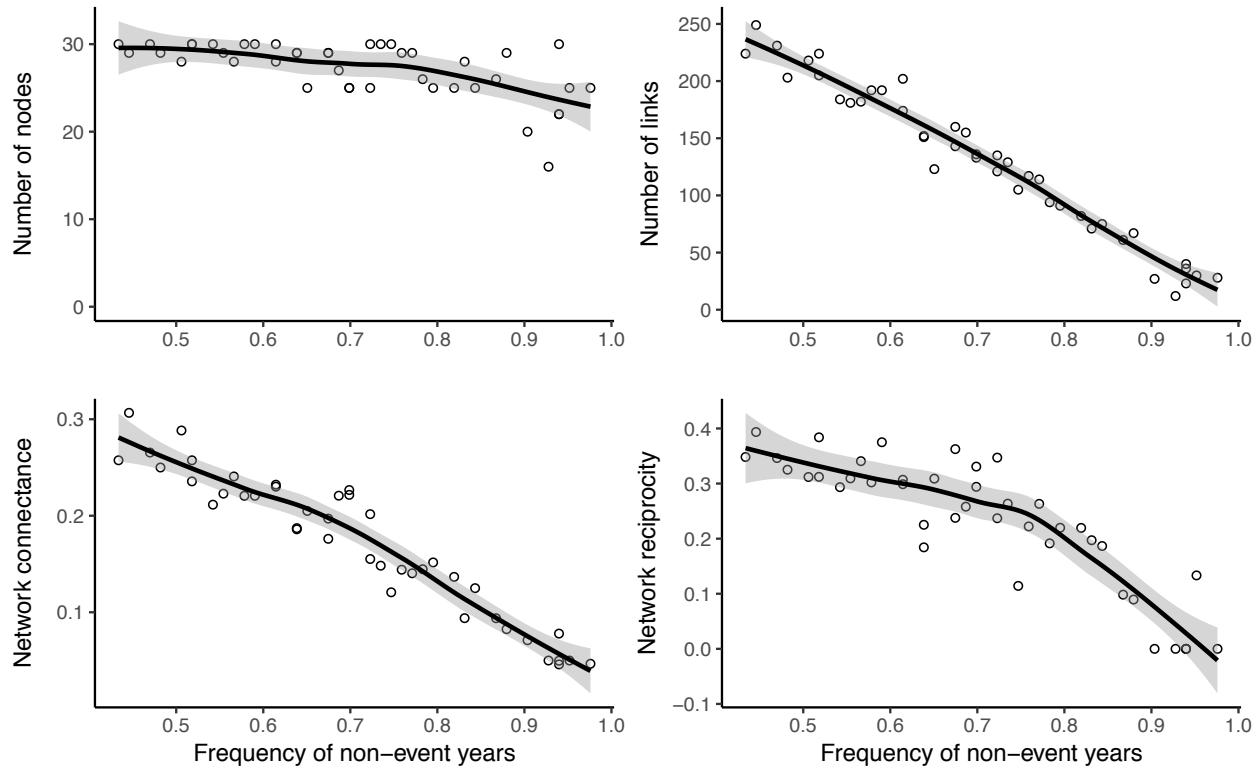
Supplementary Figure 3: Full spectrum of ecological networks ranging from extreme drought to extreme flood scenarios, showing bidirectional relationships only. The natural flow regime (56.6% flood years) is shown in the box. Nodes on the outside of the circle represent the 30 members of the network (5 guilds, 6 stage classes; color coded by guild), and links represent significant interactions (see Table 1). Node size increases with the abundance of that stage class. Node order is consistent across networks and all nodes are shown, even if unconnected. The labels on the nodes represent the guild and its stage class (1-6). HT: hydroriparian pioneer trees; XS: xeroriparian pioneer shrubs; HS: hydroriparian pioneer shrubs; MM: mesoriparian meadow species; DS: desert shrubs. Each network represents an approximately 1.5% change in the frequency of flood and drought years.



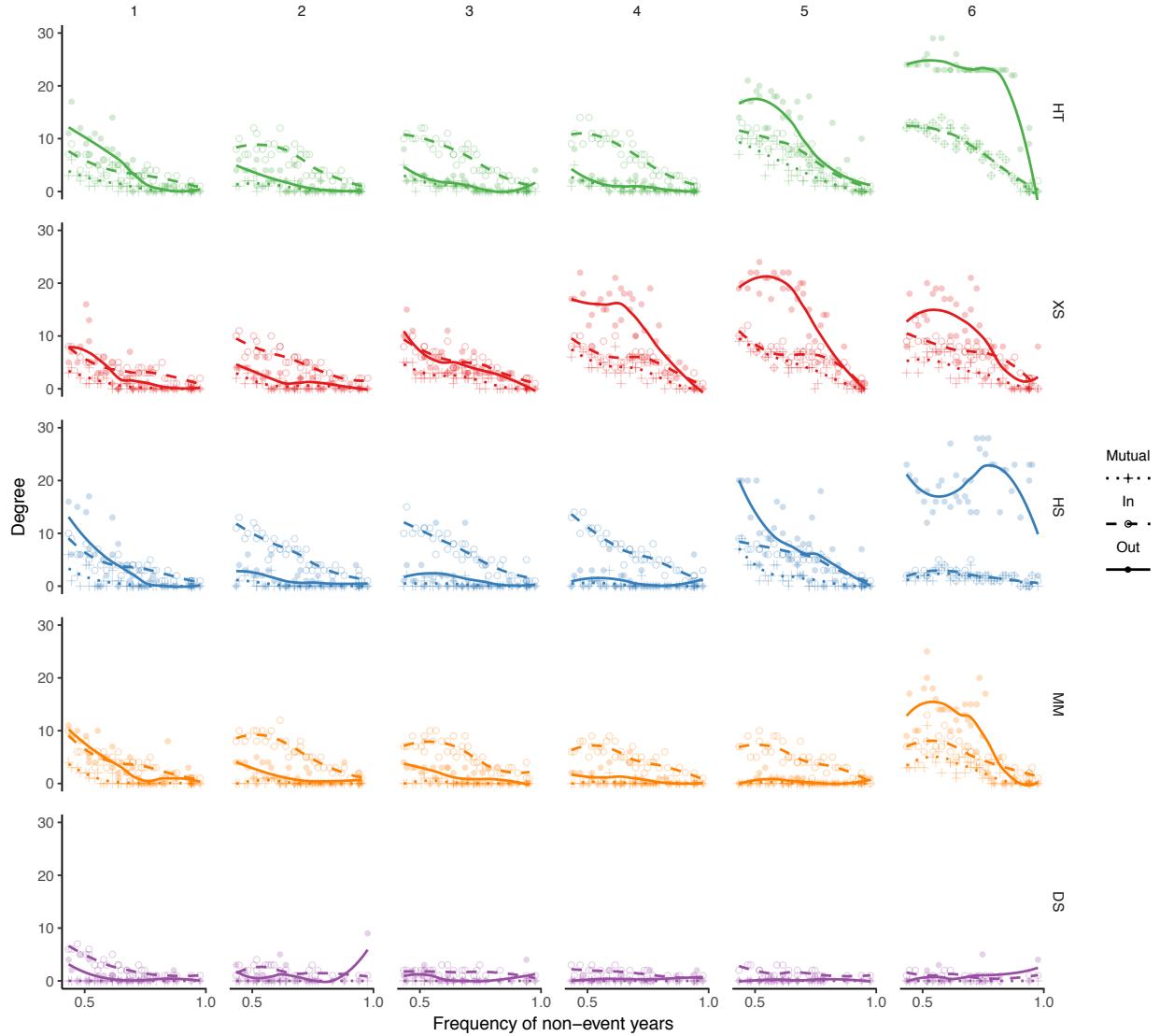
Supplementary Figure 4: Full spectrum of ecological networks ranging from the natural flow regime to 100% non-event years (flow homogenization scenario), showing directional links. The natural flow regime (56.6% flood years) is shown in the box. Nodes on the outside of the circle represent the 30 members of the network (5 guilds, 6 stage classes; color coded by guild), and links represent significant interactions (see Table 1; width = relationship strength; color matches source node; links are directional). Node size increases with the abundance of that stage class. Node order is consistent across networks and all nodes are shown, even if unconnected. The labels on the nodes represent the guild and its stage class (1-6). HT: hydriparian pioneer trees; XS: xeroriparian pioneer shrubs; HS: hydriparian pioneer shrubs; MM: mesoriparian meadow species; DS: desert shrubs. Each network represents an approximately 1.5% change in the frequency of non-event years.



Supplementary Figure 5: Full spectrum of ecological networks ranging from the natural flow regime to 100% non-event years (flow homogenization scenario), showing bidirectional relationships only. The natural flow regime (56.6% flood years) is shown in the box. Nodes on the outside of the circle represent the 30 members of the network (5 guilds, 6 stage classes; color coded by guild), and links represent significant interactions (see Table 1). Node size increases with the abundance of that stage class. Node order is consistent across networks and all nodes are shown, even if unconnected. The labels on the nodes represent the guild and its stage class (1-6). HT: hydroriparian pioneer trees; XS: xeroriparian pioneer shrubs; HS: hydroriparian pioneer shrubs; MM: mesoriparian meadow species; DS: desert shrubs. Each network represents an approximately 1.5% change in the frequency of non-event years.



Supplementary Figure 6: Summary metrics of full ecological networks spanning the spectrum of flow from the natural flow regime to 100% non-event years (flow homogenization scenario). Trend lines represent locally weighted smoothing (LOESS) fits, accompanied by 95% confidence intervals (shaded area). Network connectance: the ratio of the number of links to the number of possible links. Network reciprocity: the proportion of mutual links. Note that network metrics were calculated only on nodes that were connected (i.e. isolated nodes in Supplementary Figure 4 were not incorporated into networks). Each point represents an approximately 1.5% change in the frequency of non-event years.



Supplementary Figure 7: Degree (number of links connected to the node) of each network node in relation to flow-state in the flow homogenization scenario. Scenarios were run from the natural flow regime to 100% non-event years. Inward, outward, and mutual relationships are shown. Stage classes are in columns and guilds are in rows. Trend lines represent locally weighted smoothing (LOESS) fits. HT: hydroriparian pioneer trees; XS: xeroriparian pioneer shrubs; HS: hydroriparian pioneer shrubs; MM: mesoriparian meadow species; DS: desert shrubs. Each point represents an approximately 1.5% change in the frequency of non-event years.

Corresponding author(s): Jonathan Tonkin

Initial submission Revised version Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see [Reporting Life Sciences Research](#). For further information on Nature Research policies, including our [data availability policy](#), see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

► Experimental design

1. Sample size

Describe how sample size was determined.

We use simulations with 1000 replicates; in "Methods: Sensitivities and network analysis" section.

2. Data exclusions

Describe any data exclusions.

No data were excluded.

3. Replication

Describe whether the experimental findings were reliably reproduced.

In our simulations, we replicated each model run 1000 times in a stochastic model.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Not relevant based on our approach. However, our modeling was based on a stochastic population model, so randomization was a key component of the process. We randomly selected years from a historical record of river flows as part of the modeling process.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

We didn't perform this type of analysis, so group allocation was not part of the process.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- | | |
|-------------------------------------|--|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement indicating how many times each experiment was replicated |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The test results (e.g. <i>P</i> values) given as exact values whenever possible and with confidence intervals noted |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A clear description of statistics including <u>central tendency</u> (e.g. median, mean) and <u>variation</u> (e.g. standard deviation, interquartile range) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Clearly defined error bars |

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

Policy information about [availability of computer code](#)

7. Software

Describe the software used to analyze the data in this study.

All analyses were performed in R.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). [Nature Methods guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No antibodies were used.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

No animals were used.

Policy information about [studies involving human research participants](#)

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.