

Leaf trait network architecture shifts with species-richness and climate across forests at continental scale

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Funding information

National Natural Science Foundation of China, Grant/Award Number: 31988102, 42141004 and 32171544; National Science and Technology, Grant/Award Number: 2019FY101300; China Postdoctoral Science Foundation, Grant/Award Number: 2020M680663 and 2021M693147

Editor: Josep Penuelas

Abstract

Variation in the architecture of trait networks among ecosystems has been rarely quantified, but can provide high resolution of the contrasting adaptation of the whole phenotype. We constructed leaf trait networks (LTNs) from 35 structural, anatomical and compositional leaf traits for 394 tree species in nine forests from tropical to cold-temperate zones in China. Our analyses supported the hypothesis that LTNs would increase in modular complexity across forests in parallel with species-richness and climatic warmth and moisture, due to reduced phenotypic constraints and greater opportunities for niche differentiation. Additionally, we found that within LTNs, leaf economics traits including leaf thickness would have central importance, acting as hub traits with high connectivity due to their contributions to multiple functions. Across the continent, the greater species richness and trait diversity observed in forests under resource-rich climates enable greater complexity in whole phenotype structure and function as indicated by the trait network architecture.

KEYWORDS

adaptation, community, forest, leaf trait network, network, photosynthesis, trait

INTRODUCTION

Leaf traits influence multiple components of plant fitness, including the responses of growth, reproduction and survival to environment, and thereby contribute mechanistically to determining species' environmental distributions (Lavorel & Garnier, 2002; Shipley et al., 2017; Stahl et al., 2014; Violle et al., 2007). As traits jointly contribute to multiple functions (La Riva et al., 2016; Sack & Buckley, 2020; Yin et al., 2018) (Figure S1),

adaptation across resource gradients results in a complex network of trait correlations (Freschet et al., 2015; Stearns, 1989) arising from, and reflecting, multiple simultaneous selective processes in adaptation or community assembly. Traits may be selected in ensemble due to structural allometries—such as when traits are linked with cell, organ or whole plant size. Alternatively, traits may be correlated due to functional trait coordination or trade-offs (Niinemets & Sack, 2006; Sack et al., 2013). For example, associations are often found among traits

related to the ‘leaf economic spectrum’ (LES) (Croft et al., 2017; Reich, 2014; Wright et al., 2004). Given the convenience of simplifying variables using principal component analyses, as exemplified by the LES, many studies have reduced multiple traits into few axes of variation (Diaz et al., 2004, 2016; Westoby et al., 2002; Wright et al., 2004). However, complex network analysis (Newman, 2003), which has been applied to gene and protein interactions (Zhu et al., 2007), social connections (Girvan & Newman, 2002), microbial communities (Wang et al., 2018), transportation systems (Wang et al., 2011), and other fields, can provide higher resolution of the correlative architecture of suites of traits across environments (He et al., 2020; Mason & Donovan, 2015; Messier et al., 2017; Poorter et al., 2014; Sack et al., 2013; Schneider et al., 2017). Here we tested hypotheses for the variation of leaf trait networks (LTNs) with unprecedented analysis of traits, species, and ecosystems across a continental-scale climatic gradient.

Several key predictions arise from ecophysiological theory for how LTN properties should vary across communities associated with contrasting climates. A looser overall trait network, i.e., with lower connectivity may indicate that plants adapt to low resource availability or stressful conditions in part by constraining certain traits associated with stress tolerance to a relatively narrow range of values, such that these traits will tend to be uncorrelated with the rest of the integrated phenotype, due to having fewer correlations with each other and/or with other traits that vary independently of stress tolerance. Additionally, we hypothesised that trait correlations would be fewer and thus LTNs more stereotypical, that is, simpler, in systems with limited resources (e.g. light, nutrients, or water) which are on average (though not universally) more species-poor than resource-rich systems, and have lower overall trait diversity on average, by analogy with the typically reported correlation of species-richness and functional trait diversity (Cornwell & Ackerly, 2009; Cornwell et al., 2006). By contrast, we expected a greater diversity in the configuration of trait correlations (Li et al., 2015; Mason & Donovan, 2015) in species of high resource systems, consistent with their contrasting strategies and occupation of more niches. Thus, based on theory, we expected simpler LTNs that are less differentiated into clusters for communities of colder or more arid biomes. Notably, this hypothesis is based on generalised patterns and exceptions are to be expected. The relationship of species-richness with resource availability may be humpbacked and some resource-poor habitats have relatively high species-richness (Petersen et al., 2020; Rice & Westoby, 1983); and stress tolerant plants may show strong variation in several traits related to specific stress tolerance mechanisms, for example, carnivory in low N soils (Paniw et al., 2017), CAM photosynthesis in arid conditions (Christin & Wood, 2016). However, the relationships of species-richness and trait variation with resource availability over a broad range

of climate types at large scales is consistent with previous reports in the literature (Chauvet et al., 2017; Cornwell & Ackerly, 2009; Kreft & Jetz, 2007; Le Bagousse-Pinguet et al., 2017).

However, the few studies that have so far tested plant trait networks have not enabled any conclusive test of these hypotheses. A recent study of 10 plant traits based on a compiled global trait database, including size-related traits and economic traits, grouping species by regions, found no overall trend in LTN modularity from tropical to polar regions, although considering only the woody species, those of polar regions did show simpler LTNs than other communities (Flores-Moreno et al., 2019). Yet, the LTN calculations in that study were subject to substantial uncertainty, as the analysis utilised a database compiled from studies using nonstandardised sampling and measurement methods, made at sites potentially far from their modeled mean climates, and involved gap filling of many missing trait datapoints, all of which would have reduced the precision of LTNs and the analysis of their potential shifts.

There has also been controversy surrounding the importance of specific traits within LTNs. Some have hypothesised that in general, leaf economics traits, including leaf thickness, would be central within LTNs. Leaf thickness contributes to multiple functional processes, being closely anatomically linked with the size of cells, the number of cell layers (John et al., 2017) and the thickness of the cuticle (John et al., 2013), and thicker leaves would be expected to have greater area-based light absorption, nitrogen allocation, water transport and carbon fluxes per leaf area (Sack et al., 2003), as well as higher leaf mass per area (LMA) (Niinemets, 2001), and thus mechanical strength (Onoda et al., 2011). However, recent studies have provided mixed evidence, with leaf thickness and water content resolved as hub traits in epiphytes and herbs in a study of 2882 species of vascular epiphytes and non-epiphytic herbs (Hietz et al., 2021), foliar nutrients (N, P, K, Mg) in the 52 tree species across 1000–3000 m elevations (Homeier et al., 2021) and stem specific length and biomass allocation traits in herbaceous perennial plants, rather than leaf economic traits including LMA and mass-based leaf nitrogen concentration, in a study did not consider leaf thickness and other associated traits (Kleyer et al., 2019).

To test these hypotheses for diverse forests arrayed along a climatic gradient, we quantified the LTNs and their parameters for 35 leaf traits of 394 tree species from the boreal zone to the tropics. We quantified five LTN-level parameters representing the network topological connectivity and complexity, and for each trait within the networks, four parameters representing trait connectivity and centrality (Table 1). We hypothesised that communities of colder or drier climates would have simpler, less dense LTNs with lower modularity, consistent with their lower species diversity, stronger constraints on given traits, and reduced possibilities for functional

TABLE 1 Parameters describing the overall topology of trait networks and the properties of nodes within networks, with explanation of the property quantified, definitions, significance, and hypotheses

Network/node parameters	Network property quantified	Parameters	Definition	Significance of a higher value	Hypotheses for overall network parameter shift from low to high climatic stress	Hypotheses for parameter values of leaf economics traits, including leaf thickness and associated traits relative to other traits
Parameters describing the overall topology of trait networks	Connectivity	Edge density (ED)	The proportion of actual connections among traits out of all possible connections	Stronger independence among traits	Decrease	na
	Connectivity	Average path length (AL)	The shortest path between each pair of nodes averaged across the network.	Stronger independence among traits	Increase	na
	Connectivity	Diameter (D)	The maximum shortest distance between any two connected node traits in the network	Stronger independence among traits	Increase	na
	Complexity	Average clustering coefficient (AC)	The average of the clustering coefficients of all traits in the network	Less pronounced division of network into subcomponents	Increase	na
	Complexity	Modularity (Q)	The difference between the fraction of connections among traits within a given module and that in a null model where connections among traits are randomly distributed	Greater tendency for traits to cluster into modules	Decrease	na
Parameters describing the properties of nodes within networks	Connectedness	Degree (k)	The number of edges that connect the focal node trait to other nodes	Greater connectivity of the focal node trait within the network—a 'hub' trait	na	High
	Connectedness	Closeness (C)	The reciprocal of the mean shortest path between a focal node trait and all other nodes in the network	Closer connection of the focal node trait with other traits in the network	na	High
	Centrality	Betweenness (B)	The number of shortest paths going through a focal node trait	Greater role of the focal node trait as 'mediator' of trait connections within the network	na	High
	Centrality	Clustering coefficient (CC)	The proportion achieved of potential connectivity among the neighbours of a focal node trait	Greater role of the focal node trait as a center of a module of traits	na	High

differentiation among niches (Table 1) (Liu et al., 2019; Yin et al., 2018). We also conducted tests of the dependency of calculated LTN parameters on the number of species in a community and on the number of traits considered, conducting simulations in which different numbers of species or traits were sampled from the total pool, hypothesising that all else being equal, LTNs for forests containing more species were more likely to resolve significant correlations, and thus to have greater connectivity, and potentially, higher complexity, as would LTNs based on a greater set of diverse traits. Furthermore, we considered the structure of trait organisation, hypothesising, by analogy to gene or protein networks (Zhu et al., 2007), that traits would assemble in LTNs according to pre-defined structural scales or functional processes—that is, leaf economics and light harvesting traits, leaf size and anatomy traits, stomatal traits, and carbohydrate and energy traits. Finally, we hypothesised that generally across communities, leaf economics traits and in particular thickness and its associated traits would have disproportionate associations with other traits, and thus special importance in LTNs, with high connectivity and centrality within modules (Table 1). Overall, we quantified the architecture of LTNs across diverse forests at the continental scale to determine the climate-dependence of the organisation of traits of biological significance.

METHODS

Site description

The north-south transect of eastern China is the 15th standard transect of the International Geosphere-Biosphere Programme and spans from a tropical rain-forest in the south to a cold-temperate coniferous forest in the north, covering almost all forest types in the Northern Hemisphere (Zhang & Yang, 1995). This transect provides a robust platform to quantify the ecological and evolutionary determinants of plant environmental distributions at a large scale. Representatives of nine common forest types were selected along the transect, from 18.7–51.8°N, ranging in mean annual temperature (MAT, °C) from –4.4–20.9°C and in mean annual precipitation (MAP, mm) from 482–2449 mm (Figures S2 and S3, and Table S1).

Field sampling

Sampling was conducted from July–August 2013, within the growing season for all the forests. In each forest, four experimental plots (30 × 40 m) were established (Li et al., 2018). We collected fully expanded, sun-exposed leaves from four mature and healthy individuals of each of 394 tree species by three methods (see Figure S4). At each

plot, soil samples (0–10 cm) were collected randomly from 30–50 points using a soil sampler (6-cm diameter), and then mixed thoroughly (Zhang et al., 2018; Zhao et al., 2016).

Leaf trait measurement

We compiled data that were previously published in separate studies on trait-environment relationships, including 35 leaf traits, representing leaf economics and light harvesting traits, leaf size and anatomy traits, stomatal traits and carbohydrate and energy traits (Table S2, including symbols and units). Given our inclusion of four chlorophyll (Chl) traits, for comprehensiveness, we tested the influence of removing Chl a and Chl b, leaving only total Chl and the Chl a/b ratio, thus reducing the number of traits from 35 to 33; all results were substantively similar, and thus all 35 traits were utilised in the analyses presented.

Leaf trait network analysis

To calculate the LTNs, leaf traits were considered as network nodes and trait correlations were considered as edges. A correlation coefficient matrix of species mean values for all leaf traits within each forest community was calculated; the strength of trait–trait relationships was described using the absolute value of Pearson correlation coefficients ($|r|$). To avoid spurious correlations among leaf traits, we set significance thresholds of $|r| > 0.2$ and $p < 0.05$ (Kleyer et al., 2019; Poorter et al., 2014) and an adjacency matrix $A = [a_{ij}]$ where $a_{ij} \in [0,1]$ was calculated by assigning 1 to relationships that were above the significance threshold and 0 to those below the threshold. These LTNs were visualized using the igraph package in R (version 3.3.1, R Development Core Team 2016).

Calculation of LTN-level parameters

To describe the overall topology of LTNs, we used three parameters to quantify the ‘connectivity’ of the LTN, that is, the overall inter-relatedness of all traits—the edge density (ED), the diameter (D), average path length (AL); and two parameters for the ‘complexity’ of the LTN configuration, i.e., the differentiation into clusters—the average clustering coefficient (AC), and the modularity (Q) (summarised in Table 1; illustrated in Figure S5 and calculated in Appendix A). LTNs with high D and AL are ‘looser’, that is, they have an overall high level of independence among traits. A high AC signifies fewer clusters, and a lower LTN complexity. The Q of the LTN represents the degree of separation among modules (Figure S6). The calculated trait modules for

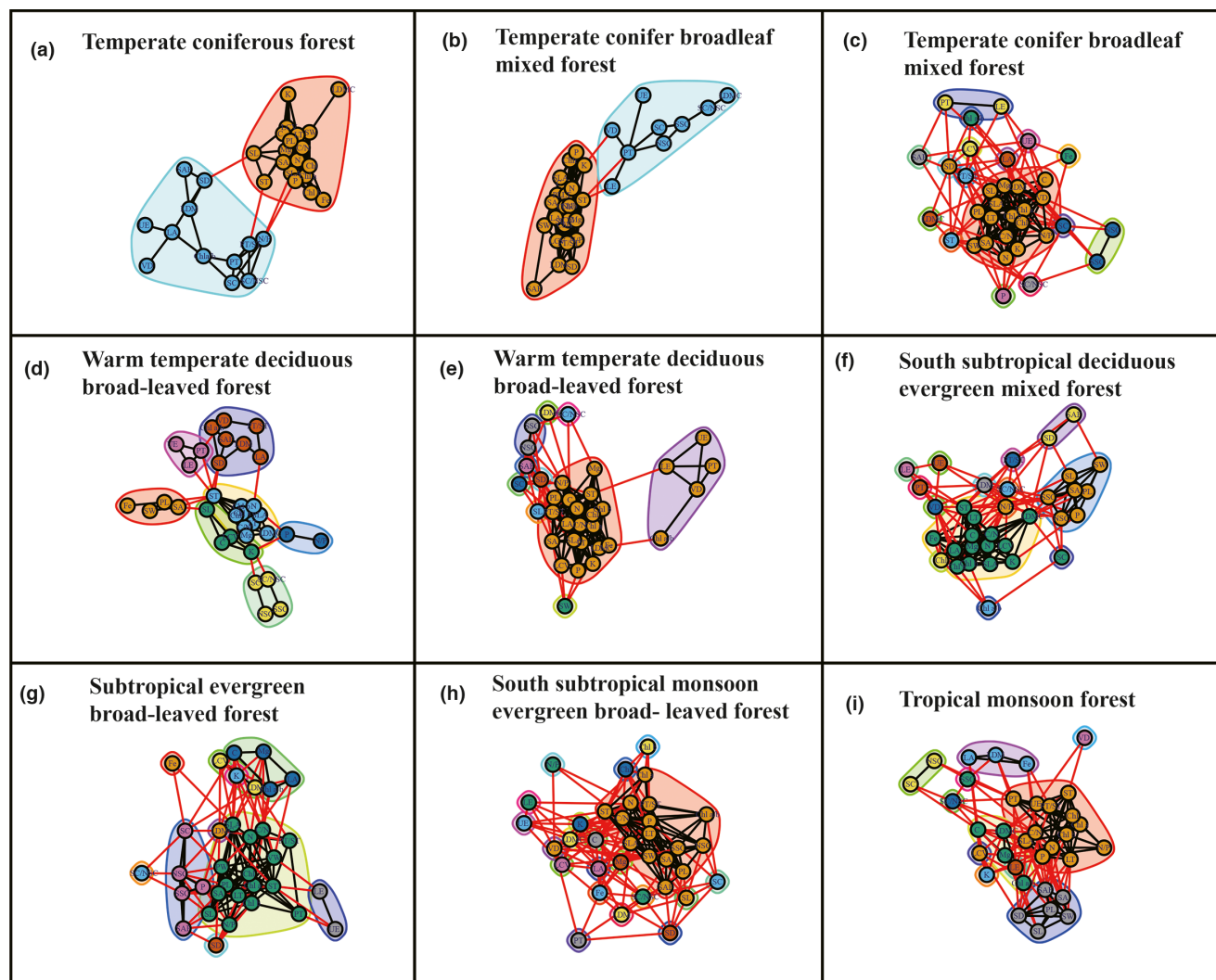


FIGURE 1 Increasing connectivity and complexity of leaf traits networks (LTNs) across forests from cold-temperate to tropical zones, with species-richness and mean annual temperature and precipitation increasing from panel (a) to (i). The nine forests distributed from North to South along the North–South Transect of Eastern China (NSTEC): (a) Huzhong (HZ), (b) Liangshui (LS), (c) Changbai (CB), (d) Dongling (DL), (e) Taiyue (TY), (f) Shennongjia (SN), (g) Jiulian (JL), (h) Dinghu (DH), and (i) Jianfengling (JF). In each panel, LTNs are divided into different modules, as indicated by node colour and coloured surrounding lines and regions. The differentiation into modules is described in ‘Calculation of LTN-level parameters’, with detailed results are shown in Table 1 and Table S3. Black lines represent edges (correlations) between nodes of the same modules, and the red lines represent edges between nodes of different modules

the nine forests are shown in Figure 1 and Table S3 and Appendix B.

Calculation of LTN trait-level parameters

To describe the importance of traits within LTNs, we used two parameters to quantify the ‘connectedness’ of each trait—the degree (k) and closeness (C); and two parameters to signify the ‘centrality’ of each trait—the betweenness (B), and the clustering coefficient (CC) (summarised in Table 1; illustrated in Figure S5, and calculated in Appendix A) (Deng et al., 2012). Traits with the highest k were ‘hub traits’, and with high C were thus closely connected to other traits, and with high B represent central, ‘mediator traits’. Moreover, the CC of traits

was considered as another index of trait centrality within the network.

Quantifying the relative connectivity of leaf traits within classes representing functional processes

To test the hypothesis that LTNs would be organised into modules corresponding to given structural scales and/or functional processes, the 35 measured leaf traits were classified into four categories defined *a priori*: leaf economics and light harvesting traits, leaf size and anatomy traits, stomatal traits, and carbohydrate and energy traits (Table S2). We quantified the organisation of traits within these classes, and whether this organisation

shifted across ecosystems. Thus we defined (1) for each trait category the ‘category trait connectivity’ as the numbers of connections relative to total number of trait-pairs within the category, and (2) the ‘overall LTN category trait connectivity’, as the overall numbers of trait correlations within categories relative to total number of trait-pairs across the whole LTN, and (3) the ‘overall LTN cross-category trait connectivity’, as the overall numbers of correlations among traits of different pre-defined categories across the whole LTN relative to the total number of trait-pairs across the whole LTN. We tested whether these ratios were significantly higher than the ratio of significant correlations relative to total correlations tested across the LTN. Furthermore, we tested whether these two parameters varied across nine forests in association with climate using least squares regression, with log-transformation.

Soil and climate data

Soil total nitrogen (STN, g kg^{-1}) was determined using an elemental analyzer (Vario Analyzer, Elementar, Germany). Climate variables, that is, mean annual temperature (MAT) and precipitation (MAP), were extracted at 1×1 km spatial resolution using the interpolation software ANUSPLIN (He et al., 2014) based on 740 climate stations of the China Meteorological Administration from 1961–2007.

Data analysis, including simulated networks to test for the intrinsic dependence of LTN parameters on species-richness and trait numbers

The LTN-level parameters and trait-level parameters, and the category and overall LTN category trait connectivities were calculated using the ‘IGRAPH’ package in R.

Ordinary least square regression was used to test the relationships of network parameters with latitude, and with climate and soil variables. Spearman rank correlation was used to quantify similarity of the rank of trait-level parameters (k , C , B and CC) among the nine sites.

To test the dependence of LTN-level parameters intrinsically on the numbers of species and numbers of traits, we conducted simulations based on the entire dataset combining species from all communities. We randomly sampled species numbers from 10 to 394, and trait numbers from 10 to 35 by sampling with replacement, and for each combination of species and trait number, 500 LTNs were determined and their LTN-level parameters were calculated. We plotted the average values of LTN-level parameters against numbers of traits and species considered.

To test whether the observed trends of LTN parameters with climate differed from those for simulations

based on sampling species sets of different sizes from a random species pool, we compared the slopes of the least square regressions of parameters versus climate for the observed parameters, the simulated parameters based on randomly sampling communities with different species richness, and tested whether the slopes overlapped in their confidence intervals.

To further test the robustness of climatic trends of LTN-level parameters, we conducted two sets of simulations. First, we tested the potential dependence of the climate association of LTN-level parameters on the number of leaf traits. Thus, for each site at their specific species richness, we subsampled 27–34 leaf traits of the 35 measured, determined LTNs, calculated ED , D , AL , AC and Q (500 iterations), then we tested the association of average simulated LTN-level parameters with climate. Second, to further clarify whether, all else being equal, the species richness would affect the climate association of LTN parameters, we used the species number of the coniferous forest, which had the lowest species number ($n = 15$), and thus randomly sampled 15 species for each of the other forests (500 iterations) and determined LTN-level parameters, then we tested the association of average LTN-level parameters with climate.

To test the generality of the importance of given traits with LTNs across forests, we tested the relationships across traits between the ‘stability’ of each LTN-trait level parameter across the nine forests and the mean value of the LTN-trait-level parameter, considering stability as the inverse of the coefficient of variation (Zhang et al., 2017):

$$stability_i = \frac{Mean_i}{SD_i} \quad (1)$$

where i is a node parameter (i.e. k , C , B or CC), and SD_i is the standard deviation of parameter i among nine sites. Traits with higher stability maintain their relative importance in the network topology more robustly across the range of forests sampled across the climatic gradient. Thus, a positive relationship across the measured traits of trait stability with the mean LTN parameter value would signify that those traits that are on average important within the LTN across the forests also tend to maintain their importance within the networks of the different forests.

RESULTS AND DISCUSSION

Latitudinal and climatic trends of leaf trait network complexity

Our analysis provided a new level of resolution of complexity in the organisation of species’ traits within an across ecosystems. Beyond quantifying the variation of the traits, or even the numbers of associations among

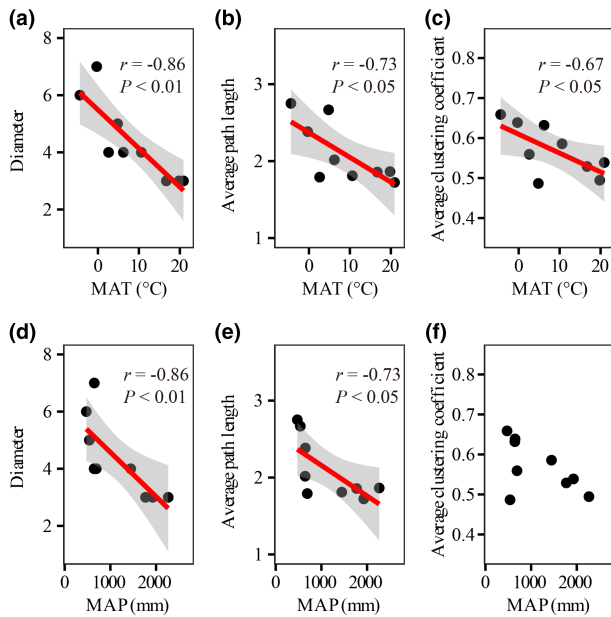


FIGURE 2 The relationships between network diameter (a and d), average path length (b and e) and average clustering coefficient (c and f) of the leaf trait networks and environmental factors across nine forests across a continental climatic gradient. In each panel, only regressions with $p < 0.05$ in the direction hypothesized are presented. The red lines were fitted using linear regression. Shaded areas indicate 95% confidence intervals. MAT: mean annual temperature; MAP: mean annual precipitation

traits, network analysis breaks new ground in the identification of configurations and modularity of these associations, and their functional categories. As hypothesised, the leaf trait networks (LTNs) shifted across forests of the Northern Hemisphere with climate, from simpler networks with lower connectivity for the coniferous forests with lower species diversity in the higher latitude cold temperate zone, to greater complexity and connectivity for tropical rainforests (Figure 1; Table S4, and Appendix B). Thus, both the diameter (D) and average path length (AL) were correlated with latitude, indicating the greater connectivity of LTNs from cold-temperate forests to tropical forests (Figure S7). These latitudinal patterns were strongly related to climate, that is, to mean annual temperature and rainfall (Figure 2), and partially with increasing soil nitrogen concentration (Figure S8). Moreover, we found that average clustering coefficient (AC) also increased with latitude (Figures S7 and S8), suggesting increasing trait differentiation into clusters within the LTNs from coniferous to tropical forests. Notably, the same climatic trends of D , AL and AC were observed when calculating LTNs for simulated forests with subsampled numbers of traits (Table S5). Across the forests, the network edge density (ED) and modularity (Q) were statistically independent of latitude, climate, and soil nitrogen concentration (Figures S9).

As hypothesised, the LTN parameters were strongly sensitive to forest species richness, when simulating forests with different species numbers drawn from the pool

of all species across the nine forests. Thus, when simulating forests with higher species numbers, the LTNs increased in edge density, and decreased in diameter, average path length and average clustering coefficient, and showed a decreased followed by an increase in modularity; all parameters eventually approached stable values, though typically only after forests were simulated with more than 10–100 species (Figures 10a–e). Similarly, when simulating forest LTNs with increasing numbers of randomly sampled traits, the edge density, the diameter, average path length average clustering coefficient and modularity decreased, though with diminishing slope, and only a weak effect of further traits above 35 (Figure S11a–e). These effects indicate that more diverse communities, with higher species numbers, are more likely to show significant correlation between traits, and thus to result in networks of greater connectivity and complexity. Further the consideration of additional traits would further resolve trait network connectivity and complexity. Notably, the specific traits considered would be important; our simulations considered randomly sampled traits across our diverse suite of measured variables. The influence of including additional traits would depend on which traits were included, and their intrinsic relationships with other traits. For example, if additional traits were included that were intrinsically highly correlated with others within the network, this would be expected to especially increase network connectivity, and also the modularity, if the newly included traits were correlated disproportionately with some but not others within the network.

We tested whether the higher species numbers in lower latitude forests could account for the observed relationships of forest LTN parameters with latitude if all species were drawn randomly from a single pool (Figure S12). Such a finding would be analogous to the well-recognised dependence of ecosystem processes on species-richness due to its representation of functional diversity, often referred to as the ‘sampling’ effect (Loreau & Hector, 2001). We tested the differences between the observed relationships of LTN parameters and climate and the trends arising due simply to species-richness in simulated forests based on randomly sampling species from the total species pool, and found that the observed trends could be explained by species richness (Figures S12–S14; Table S6). To further test the role of species richness in determining variation in LTN parameters across forests across latitudes, we sampled randomly from each forest 15 species, that is, the number of species in the most species-poor community, and calculated LTNs for each forest. This simulation reversed the climatic trend for diameter, modularity, and lower edge density (Figure S15). This analysis confirmed that species-richness is the principal mediator of the relationship of LTN parameters to climate. Therefore, the assembly of fewer species in stressful ecosystems, within given niches, and overall, can explain the effect of climate on LTN parameters,

reflecting stronger constraints on trait diversity and thus on phenotype complexity. This mechanism for shifting trait architecture with climate is analogous to the ‘sampling’ effect of increasing functional diversity with higher species richness explaining higher productivity in more species-rich ecosystems (Cornwell & Ackerly, 2009; Cornwell et al., 2006). Thus, as shown by our empirical data and simulations, the numbers of tree species of given forests across a latitudinal gradient are in the range that will importantly influence LTN parameters, with more species-rich forests showing more connected and complex networks. These findings indicate that the shift of LTN parameters with climate can be explained at least in part as a consequence of the increase in functional diversity in parallel with greater species richness.

We found strong evidence for the organisation of traits by functional class, similarly to gene or protein systems (Zhu et al., 2007), as shown by our tests of ‘category trait connectivity’. Thus, across all forests, the category trait connectivity was higher than the between-category trait connectivity (paired *t*-test; $p < 0.05$). Furthermore, the connectivity of traits within categories varied across ecosystems (Table S7). This analysis resulted in the same empirical tendency as the test for LTN complexity (Q and AC) across forests, that is, supporting greater trait complexity in LTNs at lower latitudes and with moister, warmer climates; the ‘category trait connectivity’ was lower in coniferous forests and higher in tropical rainforests (log-transformed data; Figure S16; Table S7). Thus, the greater complexity of LTNs at lower

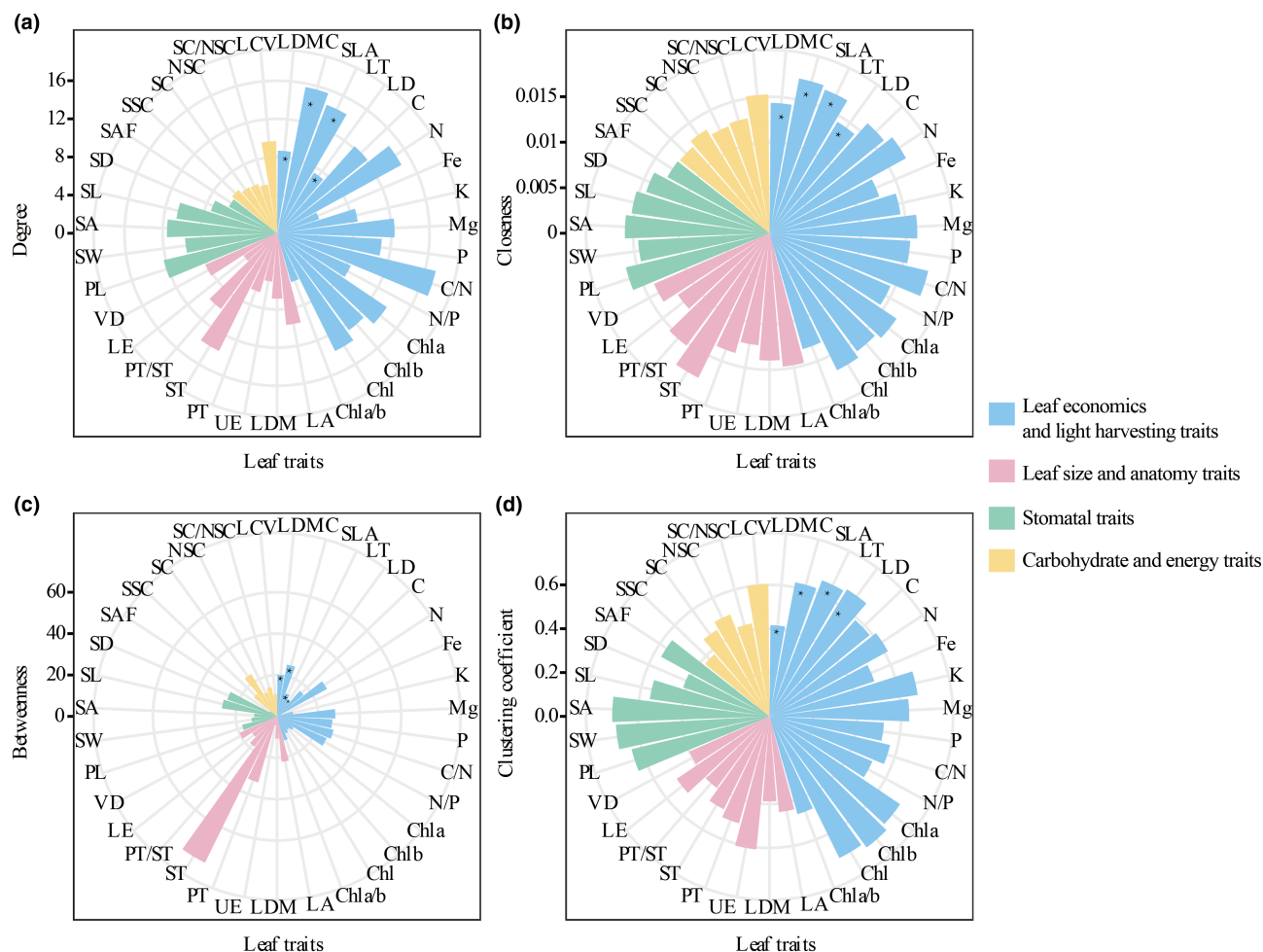


FIGURE 3 Variation in leaf trait network trait-level parameters among nine forests across a continental climatic gradient. Abbreviations: LDMC, leaf dry matter content; SLA, specific leaf area; LT, leaf thickness; LD, Leaf tissue density; C, leaf carbon concentration; N, leaf nitrogen concentration; Fe, leaf iron concentration; K, leaf potassium concentration; Mg, leaf magnesium concentration; P, leaf phosphorus concentration; C/N, the ratio of carbon to nitrogen concentration; N/P, the ratio of nitrogen concentration and phosphorus concentration; Chl a, leaf chlorophyll a concentration; Chl b, leaf Chlorophyll b concentration; Chl, total chlorophyll concentration; Chl a/b, The ratio of Chl a to Chl b; LA, leaf area; LDM, leaf dry mass; UE, upper epidermal cells width; PT, palisade tissue thickness; ST, sponge tissue thickness; PT/ST, the ratio of palisade to spongy tissue thickness; LE, lower epidermal cells width; VD, vessel diameter. PL, stomatal pore length; SW, stomatal width; SA, stomatal area; SL, stomatal length; SD, stomatal density; SAF, stomatal area fraction; SC, starch concentration; SSC, soluble sugar concentration; NSC, Non-structural carbohydrate concentration; SC/NSC, the ratio of SC to NSC; LCV, leaf caloric value. * The traits belong to multiple categories traits. LDM, SLA, LT, and LD are both in categories of leaf economics and light harvesting traits and leaf size and anatomy traits

latitudes was resolved whether considering trait clustering using the designation of network modules ‘blind’ to a priori trait categories, or considering trait clustering into pre-determined trait functional classes. The greater association of traits within categories in warmer, wetter climates can be explained by the higher species numbers and associated wider ranges of trait values (Sack et al., 2003). Notably, even if communities varying in climate had comparable species richness, the adaptation and plasticity of traits for tolerance in stressful environments might also cause shifts in networks with climate analogous to those we found in this study, a hypothesis requiring testing in future studies.

Identification of connected and central traits within LTNs

We found strong support for the hypothesis that certain traits are disproportionately important in their associations with other traits. We tested for generality in the importance of given traits within the LTNs across the diverse forests, that is, the traits’ connectedness and centrality. Traits with high degree (k) and closeness (C) can be considered ‘hub traits’ within the networks. As hypothesised,

leaf economics traits, including leaf thickness and associated traits (i.e. the thickness of spongy mesophyll, ST and specific leaf area, SLA), were especially important as LTN hubs, with higher k and C , as expected from their allometric associations, relationships with biochemical traits relating to carbon or water fluxes per area, and leaf economics. Thus, area-based foliar concentration of nitrogen (N) and chlorophyll a and b (Chl a and Chl b) would be positively related to leaf thickness and/or negatively related to SLA (Figure 3). Leaf thickness-related traits including spongy and palisade thickness, also had higher betweenness (B); such traits would act as bridges in the trait network by connecting other traits belonging to different modules (Figure 3).

Across the forests, the traits maintained their hierarchy in terms of k and C in the LTNs, indicating the existence of conserved elements of LTN architecture across the climatic gradient. Thus, leaf thickness and nitrogen concentration were typically high in degree and closeness, and upper and lower epidermal cell thickness were typically low in these parameters. By contrast, trait rankings for betweenness and clustering coefficients were not well maintained across forests (Tables S8–S11). Further, across forests, the “stability” of LTN trait-level parameters, i.e., the traits’ general importance in the

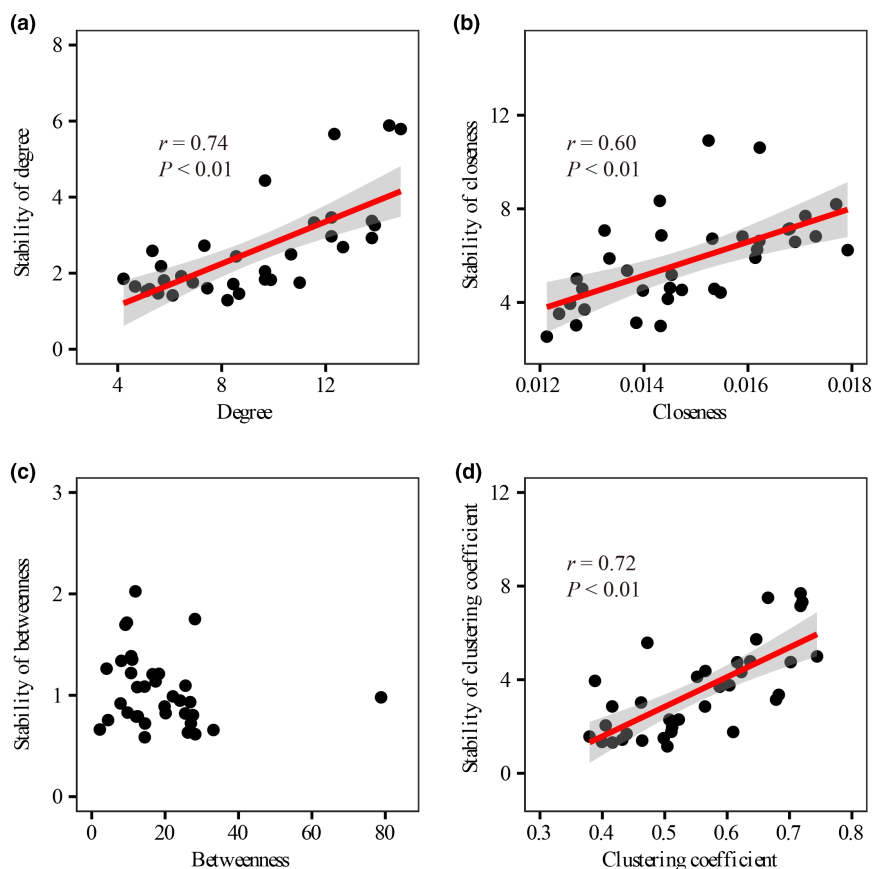


FIGURE 4 Relationship between the degree (a), closeness (b), betweenness (c), clustering coefficient (d) and their stability in the leaf trait networks across nine forests across a continental climatic gradient. The red lines were fitted using linear regressions, and only significant regressions ($p < 0.05$) are shown. Shaded areas indicate 95% confidence intervals

networks for the nine forests, was related to the average importance of the trait in the network; the stability coefficient for given parameters was related to the mean parameter value for k , C , and CC , though not B (Figure 4). Thus, traits with higher importance in the LTN more consistently hold their place as LTNs shift along climatic gradients. As traits with lower values of LTN trait-level parameters are more variable in their roles within the network, we concluded that the connections among key traits and between key traits and other traits are more robust than those among peripheral traits.

Implications and challenges in assessing LTNs

The adaptation and/or assembly of multiple plant traits across environments was clarified with their correlative architecture quantified at high resolution by the analysis of LTNs. In recent years, trait correlations have become a key focus in assessing global trends in plant adaptation, leading to improved parameterisation of ecosystem and earth system models, although traditional methods, such as principal component analysis (Diaz et al., 2016) or focusing on well-recognised suites of specific traits, such as the leaf economics spectrum tend to identify very few dimensions of trait covariation (Wright et al., 2004). Though numerous studies have explored how traits correlate with climate across gradients, few tests have been made of how the strength of trait correlations individually, or as a network, shift across climates. As a supplement to other trait analyses (such as bivariate correlations or principal components analyses), LTNs enable important additional clarity and improved consideration of the wider range of key plant traits. LTNs have been used in several previous studies to conceptualise and visualise the relationship among plant traits (Mason & Donovan, 2015; Poorter et al., 2014; Sack et al., 2013; Schneider et al., 2017), and very recently parameters been extracted to quantify trait network patterns (Flores-Moreno et al., 2019; Kleyer et al., 2019; Messier et al., 2017). Our study shows that across the continent, the architecture of the phenotype shifts to greater complexity in resource-rich climates, reflecting the greater species richness and functional diversification. This result indicates a great potential of trait network parameters to highlight the shifts in the total phenotype across communities, in response to climate, and highlighted the degree that overall phenotype integration at community scale it is modulated by species richness.

Leaf economic traits, and in particular leaf thickness-related traits, have been previously hypothesised as hub traits within LTNs (Flores-Moreno et al., 2019; Niinemets & Sack, 2006; Sack et al., 2003), and our study provides a unique test supporting this hypothesis. This result was not found in networks constructed using traits across organs and life stages (Kleyer et al., 2019); our positive result suggests that resolving hub traits depends on the inclusion

of sufficiently numerous and diverse organ-specific traits sampled across levels of organisation (i.e. anatomy, composition, pigments, etc.). Additional methodological improvements in our study also would contribute to the resolution of specific important hub traits, including sampling many species with standard methodology.

Analysis of LTNs strongly supported the overall hypothesis that relatively species-poor communities of colder and more arid climates represent a narrower range of integrated phenotypes, resulting in a less connected and simpler trait network topology, with fewer correlations among traits. By contrast, communities in the tropics showed greater LTN connectivity and complexity, related to greater species numbers, consistent with greater functional diversity and differentiation into a greater number of niches. Notably, our finding contrasted with that of a previous study based on a compiled global database, which reported no shift of trait network parameters with climate (Flores-Moreno et al., 2019). That study had focused on few plant size-related traits and economic traits across environmental gradients and growth forms; while these types of traits are widely measured and available due to their important functions, their inter-relationships tend to be conservative. Our findings suggest that trait network analysis has greater potential to resolve variation among ecosystems when including traits that capture a wide range of aspects of the phenotype that are important to function and to ecological adaptation. Future studies can extend the trait network approach to other traits of other organs, and can test LTN parameters will shift with the consideration of intraspecific trait variability. This approach has great potential for including additional complexity, and showing how it scales up to influencing trait covariation at high resolution. We note that trait network topology and calculated parameters will depend on the traits included. The selection of traits may influence the structure of LTNs, to an extent that requires further study in future work. It is important to evaluate the ability of LTNs to disentangle the importance of given traits and their topology for contrasting types of traits sets. A leaf trait is more likely to be a hub if many leaf traits are included in a network; likewise, an economic trait is more likely to be a hub if many economic traits are included. Thus, a design such as used in this study, focusing on traits across levels of organisation for a key organ, would be well-suited to identify key central traits, and the overall network topology across diverse traits.

Our study resolved for the first time climatic trends in LTNs. We attribute this result to the greater quantity of data than previous analyses, the matching of measured plants with their native climate, and the standard measurement protocols at all sites. While extensive plant trait databases exist, such as TRY (<https://www.try-db.org>) (Kattge et al., 2011, 2020), and sPlot database (<https://www.idiv.de/en>) (Bruehlheide et al., 2018, 2019), the usefulness of trait networks will motivate greater efforts for well-matched

consistently collected data, such that these databases will fulfil their promise for constructing trait networks globally. Indeed, while our study highlighted shifts with climate, this transect of forests across the continent mainly varies in temperature (Zhang & Yang, 1995), and further LTN studies extending across a greater number and types of communities occupying different climates are needed to further test the generality of hub traits and to disentangle associations of LTN parameters with temperature from other environmental conditions.

ACKNOWLEDGEMENTS

This work was supported by National Natural Science Foundation of China [31988102, 42141004, 32171544], the National Science and Technology Basic Resources Survey Program of China [2019FY101300], and the fellowship of China Postdoctoral Science Foundation [2020M680663, 2021M693147].

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

AUTHOR CONTRIBUTION

N.H. planned and designed the research; Y.L., C.L., L.X., M.L. and J.Z. conducted fieldwork and collected data; C.L., L.S. and Y.L. analysed data and wrote the manuscript; N.H., Y.L., L.S. and C.L. revised the manuscript.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ele.14009>.

DATA AVAILABILITY STATEMENT

Data supporting the results are available in the Figshare <https://doi.org/10.6084/m9.figshare.19426334.v1>.

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SUPPORTING INFORMATION

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How to cite this article: Li, Y., Liu, C., Sack, L., Xu, L., Li, M., Zhang, J. et al. (2022) Leaf trait network architecture shifts with species-richness and climate across forests at continental scale. *Ecology Letters*, 25, 1442–1457. Available from: <https://doi.org/10.1111/ele.14009>

APPENDIX A

CALCULATION OF LTN-LEVEL AND TRAIT-LEVEL PARAMETERS

Calculation of LTN-level parameters

The edge density (*ED*) describes the density of the connected edges between nodes in a network, that is, the proportion of actual connections among traits out of all possible connections.

$$ED = \frac{2L}{n \cdot (n - 1)} \quad (A1)$$

where *L* is the number of edges in the network, and *n* is the number of node traits.

D refers to the maximum shortest path distance between any two traits, and *AL* the mean shortest path between all traits. A path is a sequence of traits, each connected by edges with the next; the shortest path is that between two traits with the fewest intervening traits.

$$D = \max\{d_{ij}\} (i \neq j) \quad (A2)$$

$$AL = \frac{1}{n \cdot (n - 1)} \sum_{i \neq j} d_{ij} \quad (A3)$$

where d_{ij} is the shortest distance between focal node trait v_i and node trait v_j , and *n* is the number of traits in the LTN. LTNs with high *D* and *AL* are ‘looser’, that is, they have an overall high level of independence among traits.

The *AC* was defined as the average of the clustering coefficient (*CCs*, see Eq. A9) of all traits in the LTN. Thus, the *AC* quantifies the average probability across each trait in the network, that the traits to which it is connected are themselves inter-connected (Armbruster et al., 2014); a high *AC* signifies fewer clusters, and a lower LTN complexity.

$$AC = \frac{1}{n} \sum_{i=1}^n \frac{2l_i}{t_i(t_i - 1)} \quad (A4)$$

where l_i is the number of links between traits that are related to trait v_i , and t_i is the number of traits that are related to trait v_i .

The *Q* of the LTN represents the degree of separation among modules, where modules are distinguished using the following iterated steps. First, we calculated the edge betweenness score of each edge, where the edge betweenness score was the number of shortest paths through a focal trait-trait connection, and the *Q* value was calculated as:

$$Q_t = \frac{\sum \left[\left(A_{ij} - \frac{k_i \cdot k_j}{2m} \right) \cdot \tau \right]}{2m} \quad (A5)$$

where *t* is the iteration number, *m* is the number of edges, A_{ij} is the element of the *A* adjacency matrix in row *i* and column *j*, k_i is the degree of *i*, k_j is the degree of *j*, and τ is 1 if *j* and *i* are in the same module and 0 otherwise. Then, we removed the edge with the highest edge betweenness score and recalculated the *Q* value, and recalculated the edge betweenness of the edges. We then repeated this process; as edges with highest edge betweenness are removed the graph becomes a dendrogram (Figure S6A). The final $Q = \max \{Q_t\}$, indicating particularly satisfactory splits (Newman & Girvan, 2004). Then the dendrogram was transformed into a modular trait network (Figure S6B). The calculated trait modules for the nine forests are shown in Figure 1 and Table S3.

Calculation of LTN trait-level parameters

To describe the importance of traits within LTNs, we used two parameters to quantify the ‘connectedness’ of each trait—the degree (*k*) and closeness (*C*); and two parameters to signify the ‘centrality’ of each trait—the betweenness (*B*), and the clustering coefficient (*CC*) (summarized in Table 1; illustrated in Figure S5) (Deng et al., 2012). For each trait, *k* is defined as the number of edges connecting to other traits (i.e. the number of significant correlations with other traits). Leaf traits with the highest *k* were considered to be ‘hub traits’ in the LTN.

$$k_i = \sum_{j \neq i} a_{ij} \quad (A6)$$

where a_{ij} is 1 given a significant relationship between traits v_i and v_j .

C is defined as the reciprocal of the mean of the shortest path between a trait and all other traits in the LTN. Leaf traits with high *C* are thus closely connected to the other LTN traits.

$$C_i = \frac{n - 1}{\sum_{j=1}^{n-1} d_{ij}} (i \neq j) \quad (A7)$$

where d_{ij} is the shortest distance between traits v_i and v_j , and *n* is the number of LTN traits.

B is defined by considering the shortest paths through edges between each trait-pair in the network, and counting the numbers of these shortest paths that pass through each given trait. Traits with high *B* represent central, ‘mediator traits’.

$$B_i = \sum_{jk} (j, i, k) \quad (A8)$$

where $\sigma(j, i, k)$ is the number of shortest paths between traits v_j and v_k across the node v_i .

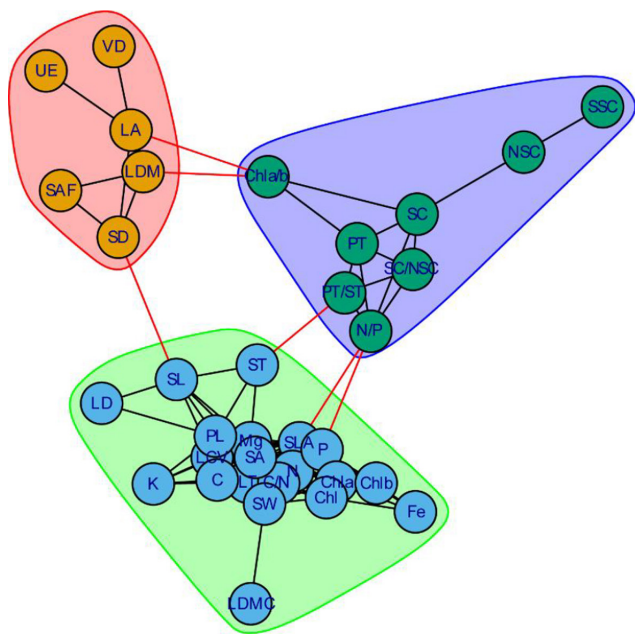
Finally, the CC of traits was considered as another index of trait centrality within the network. The CC of a trait represents the degree of inter-connectivity of the traits to which it is connected; if the traits to which the focal trait are connected are themselves fully connected, then the CC of the focal trait is 1, whereas a focal trait that is connected to traits that are not themselves inter-connected has a CC of 0.

$$CC_i = \frac{2l_i}{t_i(t_i - 1)} \quad (A9)$$

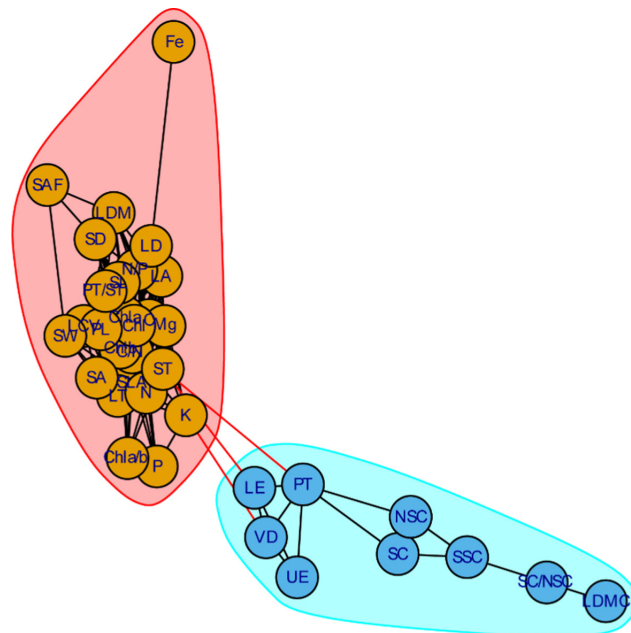
where l_i is the number of links between traits connected to focal trait v_i , and t_i is the number of traits connected to focal trait v_i .

APPENDIX B

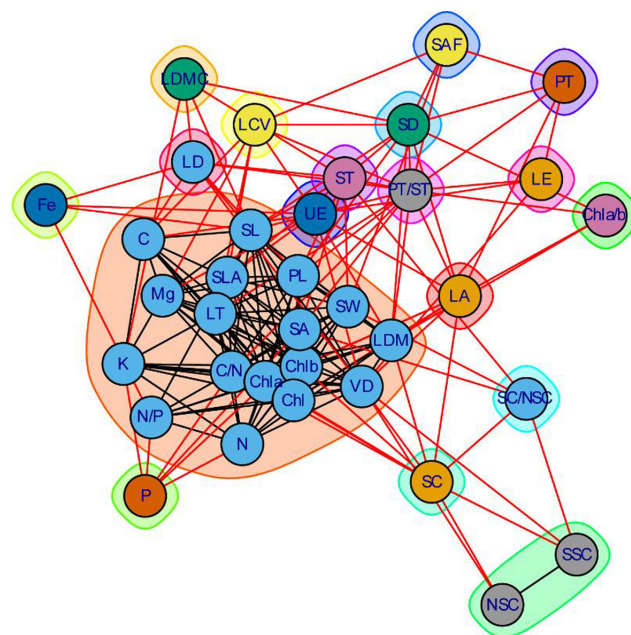
LEAF TRAIT NETWORKS OF NINE SITE



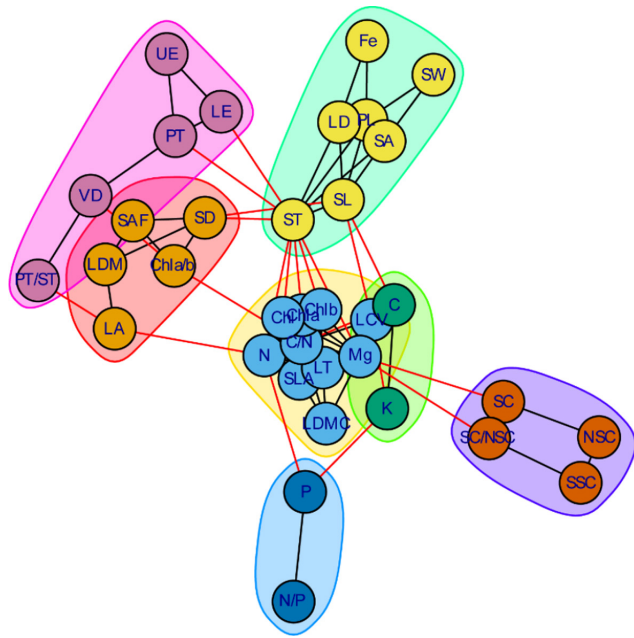
1. Leaf traits network (LTN) of Huzhong



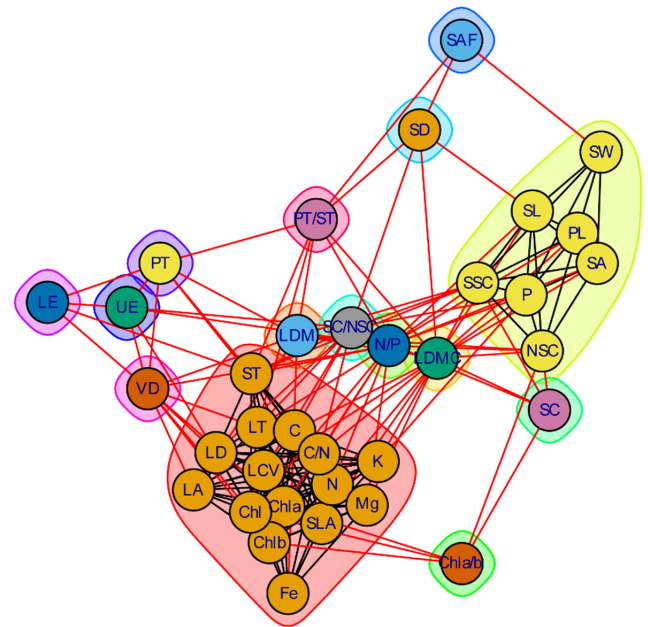
2. Leaf traits network (LTN) of Liangshui



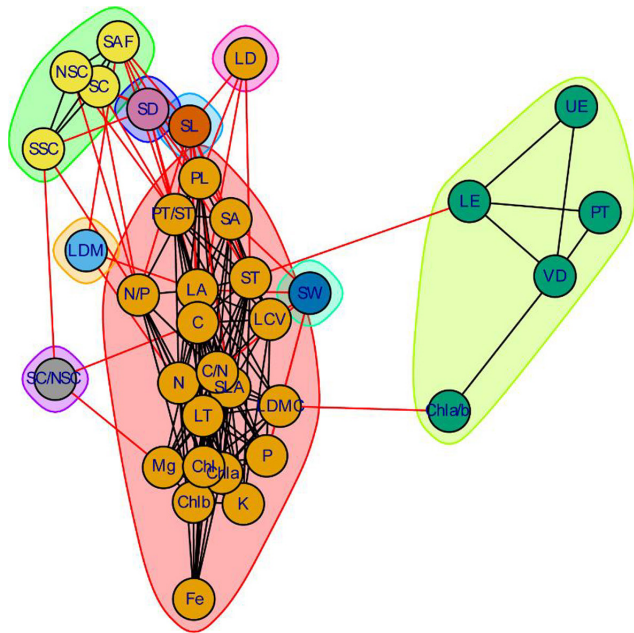
3. Leaf traits network (LTN) of Changbai



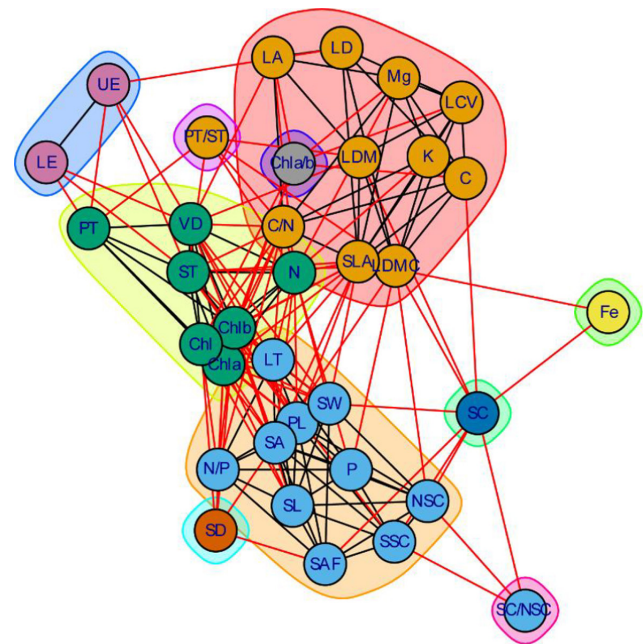
4. Leaf traits network (LTN) of Dongling



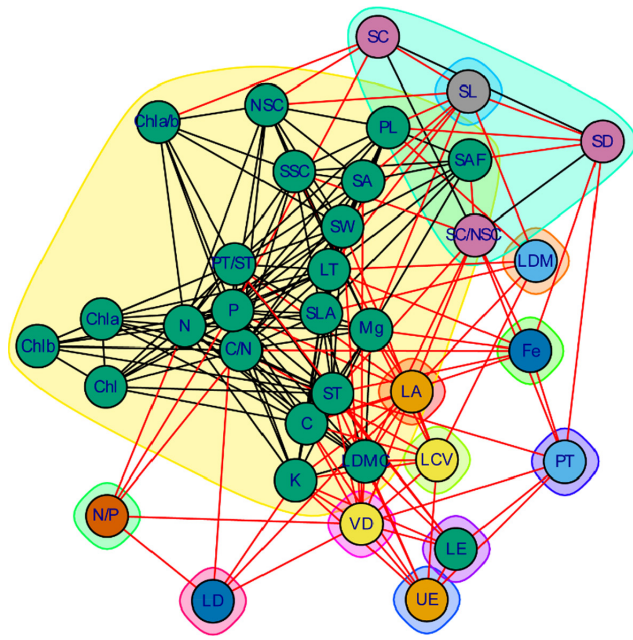
6. Leaf traits network (LTN) of Shennongjia



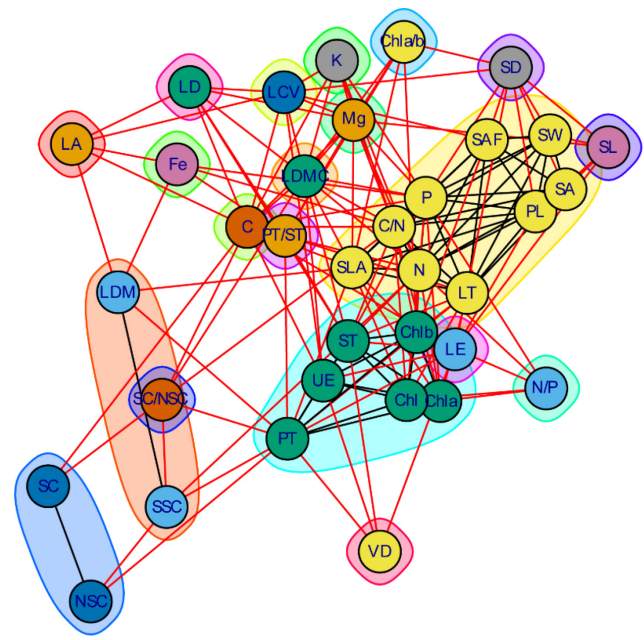
5. Leaf traits network (LTN) of Taiyue



7. Leaf traits network (LTN) of Jiulian



8. Leaf traits network (LTN) of Dinghu



9. Leaf traits network (LTN) of Jianfengling