Appendix A: Examples of the variability in community and individual plant attributes in Emas National Park, central Brazil.



a) A woodland cerrado (cerrado *sensu stricto*) six months after a fire, with several top-killed trees and a developed layer of resprouting vegetation; b) one of the sampled closed forests; c) a dense woodland cerrado (cerrado denso); d) one example of a typical thick-barked species found in open communities (Anadenanthera peregrina (Benth.) Reis, Fabaceae); e) a transitional zone between dense savannas and forests; f) a typical open savanna at the early rainy season, with tall flammable grasses and small trees and shrubs. Photo credits: Vinícius Dantas (a, e), Gabriela Sartori (b), Vivian Cadry (c), Juli Pausas (d), Felipe Noronha (e) and Alessandro Favari (f).

Appendix B. Detailed description of the field and laboratory methods

Field sampling

We estimated bark thickness in the field by measuring with a caliper the depth of penetration of a knife inserted into five haphazardly selected points in the stem at approximately 0.50 m from the ground. We estimated the height of tall trees using a four meter tall aluminum ladder and graduated fiberglass pole; when this was not sufficient, we climbed the trees with a single rope technique and measured the distance from a given point in the canopy to the upper photosynthetic tissue and from this point to the ground using a graduated rope and the above-mentioned fiberglass pole. We measured leaf toughness in the field on five completely expanded and hardened mature leaves, haphazardly selected from the outer canopy, with no sign of herbivory or pathogens (Cornelisen et al. 2003). We measured leaf toughness by drilling each leaf on both sides of the mid-rib with a cone tip of a force gauge penetrometer (Chatillon DFE 010, AMETEK, Berwyn, USA). To collect leaf samples from the canopy of very tall trees, we used a pruner with a fiberglass pole that cut branches of trees up to 11 meters tall; the four meter tall aluminum ladder was also used (Appendix A-b). When those were insufficient, we climbed trees, remove a large branch from the canopy with a pruner or a rope, and collected the material from this sub-sample. In each plot, we collected five topsoil samples (0-5 cm deep; Ruggiero et al. 2002) at each corner of the square plot and at the center.

Plant functional traits

We calculated bark thickness per stem diameter by dividing the mean bark thickness, based on the five field measurements for each individual, by the stem diameter of the tree. We measured wood density from branches collected as close as possible to the main stem following Cornelisen et al. (2003). After removing all the bark of the wood samples, we estimated volume of the wood sample by measuring the diameter and the length of the branch with a caliper and estimating the volume of a cylinder. We obtained wood density by dividing a branch's oven-dried mass (at 80°C for 72 h) by its volume. Wood density is associated with vegetative recovery ratios and mechanical strength (Enquist et al. 1999, Curran et al. 2008). Thus, low woody density is associated with high rates of vegetative recovery whereas high woody density represents high mechanical strength. We estimated leaf toughness per individual by calculating a mean value from the 10 field measurements (see field sampling). Leaf toughness is related to nutrient strategy and resistance to herbivory (Craine 2009). We estimated specific leaf area in five leaves per individual on completely expanded and hardened mature leaves, haphazardly selected from the outer canopy (Cornelisen et al. 2003). We estimated leaf area from scaled digitized images of fresh leaves using the software Image J (Rasband 2004). Specific leaf area was estimated by dividing the leaf fresh area by its oven-dried (80°C for 72 h) mass. Leaf nutrients were measured in a sample of approximately 100 g of leaf collected with the same criteria as above. Leaf nitrogen and phosphorus concentration were determined using colorimetry and emission spectrometry (induced argon plasma), respectively, following Jørgensen (1977). Leaf potassium concentration was determined using atomic absorption, following Zagatto et al. (1979). For each trait, we calculated mean trait values per plot to scale up traits measured at the individual plant level to the community level.

Soil data

We sent composite soil samples from each plot to the Soil Science Laboratory at the University of São Paulo for chemical analyses. We determined soil organic matter content (OM) and the concentration of available phosphorus (P), total nitrogen (N), cations (Ca, Mg, K) and aluminum (Al). The chemical analyses followed the methods proposed by Raij et al. (1987), Embrapa (1997) and Silva (1999). Organic matter was determined by organic carbon oxidation with potassium dichromate and subsequent potassium dichromate titration with ammonic ferrous sulfate, using 0.5 g of soil and

10 ml of potassium dichromate solution. A correction factor (1.33) was used to compensate partial carbon oxidation. Total nitrogen was determined by digestion with H2SO4, followed by distillation with NaOH, using from 0.5 to 1 g of soil, 1 g of H2SO4 and 15 ml of NaOH. Available phosphorus was determined by spectrophotometry after anion exchange resin extraction, using 2.5 cm3 of soil. The sum of bases was calculated as the sum of potassium, calcium, and magnesium, whereas aluminum saturation was calculated as a percentage in relation to the sum of bases plus Al⁺³, K⁺, Ca⁺², Mg⁺² and Al⁺³ were extracted with 1 M KCl, using 10 cm3 of soil and 100 ml of solution. Potassium, calcium, and magnesium were then determined by an EDTA complexometry. Aluminum was determined by NaOH titration.

Diversity indices

We measured both alpha and beta diversity, and we considered traditional (non-phylogenetic) as well as phylogenetic-based indices. Alpha diversity indices enable the detection of shifts along the CCI gradient in fine scale taxa co-occurrence (community level). We used species richness, Shannon's diversity index, and mean phylogenetic distance (MPD; Webb *et al.* 2002) as measures of alpha diversity. Beta diversity measures are useful for identifying switching points in community composition at the landscape scale (Graham and Fine 2008, Beselga *et al.* 2010, Ives and Helmus 2010). Furthermore, shifts in phylobetadiversity associated with changes in abiotic conditions and plant traits are expected to give insights into the patterns of trait evolution among species (Graham and Fine 2008). Thus, we used phylogenetic community distance (PCD; Ives and Helmus 2010), Euclidean community dissimilarity (both abundance-weighted and based on species presence/absence) and Jaccard's beta-diversity (calculated as the sum of the two components, the spatial turnover, and nestedness; Beselga *et al.* 2010, Ives and Helmus 2010). We computed these beta diversity indices as cumulative values along the CCI gradient to search for critical switching points in diversity or phylodiversity associated with community closure. To calculate phylogenetic alpha and beta diversities, we constructed a phylogenetic tree as described below.

Phylogenetic tree

To calculate phylogenetic alpha and beta diversities, we constructed a phylogenetic tree for all the collected species using Phylomatic software, a phylogenetic database, and a toolkit for building angiosperm phylogenetic trees (Webb et al. 2008). We first assembled an initial tree using the Phylomatic software, which was based on APG III (2009); we subsequently solved polytomies by consulting available recent phylogenetic information for Myrtaceae (Costa 2009), Fabaceae (Simon et al. 2009), Rubiaceae (Bremer 2009) and Malpighiales (Karotkova et al. 2009, Bell et al. 2010). We also dated undated nodes based on Bell et al. (2010). The remaining undated nodes were evenly spaced using the branch length adjustment algorithm (BLADJ) available in Phylocom (Webb et al. 2008).

References

- APG III. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botannical Journal of the Linnean Society 161:105-121.
- Bell, C.D., D. E. Soltis, and P. S. Soltis. 2010. The age and diversification of the angiosperms rerevisited. American Journal of Botany 97:1296-1303.
- Beselga, A. 2010. Partitioning the turnover and nestedness components of beta diversity. Global Ecology and Biogeography 19:134-143.
- Bremer, B. 2009. A Review of Molecular Phylogenetic Studies of Rubiaceae. Annals of the Missouri Botanical Garden 96:4-26.
- Cornelissen, J. H. C., S. Lavorel, E. Garniel, S. Díaz, N. Buchmann, D. E. Gurvichm, P. B. Reich, H. ter Steege, H. D. Morgan, M. G. A. van der Heijden, J. G. Pausas, and H. Poorter. 2003. A

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handbook of protocols for standardized and easy measurement of plant functional traits worldwide. Australian Journal of Botany 51:335-380.

- Costa, I. R. 2009. Estudos evolutivos em Myrtaceae: aspectos citotaxonômicos e filo-genéticos em Myrteae, enfatizando Psidium e gêneros relacionados. PhD Thesis. Universidade de Campinas, Campinas, São Paulo, BR.
- Craine, J. M. 2009. Resource strategies of wild plants, 149-250. Princeton University Press, Princeton, New Jersey, USA.
- Curran, T.J., L.N. Gersbach, W. Edwards, and A.K. Krockenberger. 2008. Wood density predicts plant damage and vegetative recovery rates caused by cyclone disturbance in tropical rainforest tree species of North Queensland, Australia. Austral Ecology 33:442-450.
- Embrapa. 1997. Manual de métodos de análise do solo. Embrapa, Rio de Janeiro city, Rio de Janeiro, BR.
- Enquist, B. J., G. B. West, E. L. Charnov, and J. H. Brown. 1999. Allometric scaling of production and life-history variation in vascular plants. Nature 401:907-911.
- Graham, C. H., and P. V. A. Fine. 2008. Phylogenetic beta diversity: linking ecological and evolutionary processes across space in time. Ecology Letters 11:1265-1277.
- Hoffmann, W. A., R. A. Dasme, M. Haridasan, M. T. Carvalho, E. L. Geiger, M. A. B. Pereira, S. G. Gotsch, and A. C. Franco. 2009. Tree topkill, not mortality, governs the dynamics of savanna–forest boundaries under frequent fire in central Brazil. Ecology 90:1326-1337.
- Ives, A. R., and R. L. Helmus. 2010. Phylogenetic metrics of community similarity. The American Naturalist 176:E128-E142.
- Jørgensen, S. S. 1977. Some methods used for routine chemical analysis. Laboratorio manual. Centro de Energia Nuclear na Agricultura, Piracicaba, São Paulo, BR.
- Korotkova, N., J. V. Schneider, D. Quandt, A. Worberg, G. Zizka, and T. Borsch. 2009. Phylogeny of the eudicot order Malpighiales: analysis of a recalcitrant clade with sequences of the petD group II intron. Plant Systematics and Evolution 282:201-228.
- Raij, B., J. A. Quaggio, H. Cantarella, M. E. Ferreira, A. S. Lopes, and O. C. Bataglia. 1987. Análise química de solos para fins de fertilidade. Fundação Cargill, Campinas, São Paulo, BR.
- Rasband, W. 2004. 'ImageJ: Image process and analysis in Java.' National Institutes of Health, Bethesda, Meryland, USA.
- Silva, F.C. 1999. Manual de análises químicas de solos, plantas e fertilizantes. Embrapa, Brasília, Distrito Federal, BR.
- Simon, M. F., R. Gretherc, L. P. Queiroz, C. Skemae, R. T. Penningtone, and C. E. Hughes. 2009. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. Proceedings of the National Academy of Sciences 48:20359-20364.
- Webb, C. O., D. D. Ackerly, and S. W. Kembel S. W. 2008. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. Bioinformatics 24:2098-2100.
- Zagatto, E.A.G., F. J. Krug, H. Bergamin Filho, S. S. Jørgensen, and B. F. Reis. 1979. Merging zones in flow injection analysis. Part 2. Determination of Ca, Mg and K in plant material by continuous flow injection - Atomic absorption and flame emission spectrometry. Analytica Chimica Acta 104:279-284.





Horizontal lines refer to values of basal areas for the different cerrado physiognomies as studied by Hoffmann et al. (2005); from *campo sujo* and *campo cerrado* (lower line) to "cerradão" forest (upper line). The equation describing the relationship between basal area and CCI is: log (Basal area) = 4.73*CCI + 0.15 (R² = 0.90; p < 0.001). The CCI is an indicator of the light environment, from open communities (CCI close to 0) to closed communities (CCI close to 1; see the Materials and Methods section in the main text for details).

Appendix D: Soil variables, leaf toughness and diversity indices along the community closure index gradient (CCI) and their breakpoints.



Soil organic matter (OM g kg⁻¹), soil N (mg kg⁻¹), soil sum of bases (mmolc kg⁻¹), leaf toughness (N), phylogenetic community distance (PCD; My) and Jaccard's beta-diversity along the community closure index gradient (CCI, 0 to 1) as examples of the threshold-type relationships found at Emas National Park in central Brazil. Vertical grey lines represent significant breakpoints (supF test).





Basal area (m².ha⁻¹) in the plots located at each side of the mean threshold along the gradient of community closure index (CCI). Open communities: CCI < 0.57; closed communities: CCI > 0.57. The CCI is an indicator of the light environment, from open communities (CCI close to 0) to closed communities (CCI close to 1; see the Materials and Methods section in the main text for details).



Mean height (m) in the plots located at each side of the mean threshold along the gradient of community closure index (CCI). Open communities: CCI < 0.57; closed communities: CCI > 0.57.