Experimental evidence that evolutionarily diverse assemblages result in higher productivity

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There now is ample experimental evidence that speciose assemblages are more productive and provide a greater amount of ecosystem services than depauperate ones. However, these experiments often conclude that there is a higher probability of including complementary species combinations in assemblages with more species and lack a priori prediction about which species combinations maximize function. Here, I report the results of an experiment manipulating the evolutionary relatedness of constituent plant species across a richness gradient. I show that assemblages with distantly related species contributed most to the higher biomass production in multispecies assemblages, through species complementarity. Species produced more biomass than predicted from their monocultures when they were in plots with distantly related species and produced the amount of biomass predicted from monoculture when sown with close relatives. This finding suggests that in the absence of any other information, combining distantly related species in restored or managed landscapes may serve to maximize biomass production and carbon sequestration, thus merging calls to conserve evolutionary history and maximize ecosystem function.

biodiversity | phylogenetic diversity | transgressive overyielding

Evidence showing that ecosystem function is positively related to the number of species in an assemblage (1–7) has profoundly changed how scientists and policy makers view the potential impact of species extinction (8, 9). However, not all species contribute equally to emergent functioning of an assemblage. How much species uniquely contribute to ecosystem functioning depends on several different aspects of species ecologies, including how variable their population dynamics are (10–12) and how susceptible they are to local extinction (13), as well as how much they overlap in their resource requirements (14, 15) and the relative distinctiveness of species in multivariate functional trait space (16, 17).

Measuring the meaningful differences in species' ecologies, across large numbers of species, has proven difficult. However, the simple assumption that the more time that has passed since two species shared a common ancestor, the higher the probability they have ecologically diverged appears to provide a powerful explanation for how biodiversity affects ecosystem function (15, 18–20). Previous analyses of the relationship between plant evolutionary history and biomass production used existing experiments that manipulated species richness. These experiments often were biased toward distantly related species, as experimenters chose to include representatives of disparate lineages (e.g., a grass or a nitrogen fixer); thus, the observed relationship between evolutionary history and productivity may have been driven by the relatively few close relatives used in these experiments (19).

Further, previous analyses of the relationship between phylogenetic diversity (PD) and ecosystem function relied on the untested assumption that distantly related species are more likely than closely related species to exhibit reduced niche overlap. The traditional explanation for richness–productivity relationships is that larger assemblages of species have a greater probability of including species that have low niche overlap and thus can access different resources (1, 21). This complementarity in resource use may be estimated by quantifying the performance of species *i*

in polyculture against the expected performance from its monocultures (22, 23). Polycultures also might appear more productive compared with monocultures via a selection effect—if highly productive species dominate the polyculture, displacing low-productivity species (22). Thus, if distantly related species show reduced resource overlap, then we should observe greater complementarity when they are combined.

Here, I report the results of a biodiversity–ecosystem function experiment that explicitly manipulated the phylogenetic distances separating species in an assemblage. A total of 97 4-m^2 plots were sown with between 1 and 4 species from a pool of 17 species (see *Materials and Methods* for details on the experiment), all of which were grown in at least three replicated monocultures. Polycultures belonged to one of the following treatments: constituent species separated by short, medium, or large phylogenetic distances (Fig. 1 *A* and *B*). These treatments created differences in plot PD, measured as the total summed phylogenetic branch lengths in an assemblage (19, 24). The experimental design fully crossed richness and phylogenetic distances. Generally, there was a positive correlation between richness and PD in the polycultures ($r = 0.51$; Fig. 1*B*), but the phylogenetic treatments maintained a high degree of variation in PD within species richness classes ($\bar{x}_{S=2} = 148.99$, SD = 123.31; $\bar{x}_{S=3} = 298.43$, SD = 132.65; $\bar{x}_{S=4} = 318.13$, SD = 152.12).

Results and Discussion

In agreement with other studies manipulating species richness, I found an effect of richness on biomass production [$\beta_{Rich} = 15.4$; 95% confidence interval (CI), 9.20–21.60; Fig. 2*A*; see *Results* for parametric statistics]. I also analyzed the effect of several different measures of diversity [incorporating relative abundances and/or phylogenetic distances (*Materials and Methods*)] and found that although several of them predicted biomass production (*SI Results*), PD was the strongest predictor ($\beta_{PD} = 0.12$; 95% CI, 0.08–0.17; Fig. 2*B*), as evidenced by model selection using Akaike weights (AW$_{Richness}$ = 0.01, vs. AW$_{PD}$ = 0.74), as well as explained variance ($R^2_{Rich} = 0.21$, vs. $R^2_{PD} = 0.28$). The effect of PD on biomass production equates to an increase of about 12 g/m^2 for every 5 My.

Despite the fact that the original design of this experiment crossed richness and phylogenetic relatedness, these two measures were not orthogonal (e.g., Fig. 1*B*). Regardless, I used a likelihood ratio test to compare a linear model including both effects, with and without an interaction term, with the PD-only model. The more complex models did not provide a better predictive ability over the PD-only model (PD vs. PD + Rich: $X^2 = 0.653$, $P = 0.420$; PD vs. PD + Rich + PDxRich: $X^2 = 0.184$, $P = 0.668$).
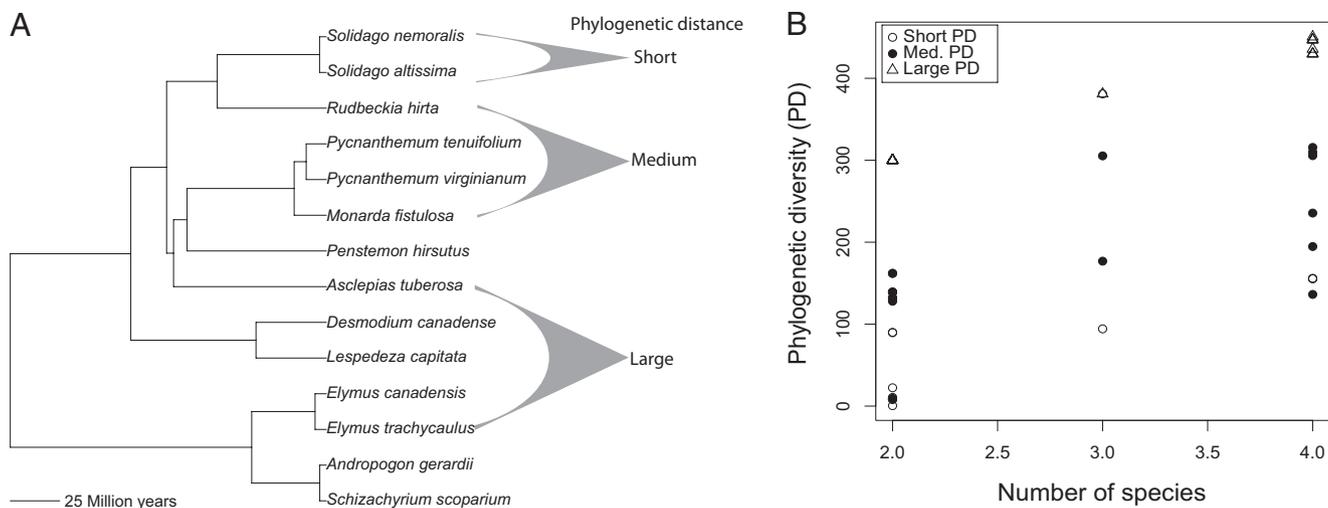
Fig. 1. (*A*) The time-calibrated molecular ML phylogeny of the species used in this experiment. The gray arrowheads indicate example two-species combinations that constitute the phylogenetic treatments. (*B*) PD across realized richness. Treatments produced a gradient in PD within each richness level.

I then estimated complementarity and selection effects following Loreau and Hector (22), and found that complementarity increased with species richness ($\beta_{Rich} = 8.80$; 95% CI, 1.10–16.51; AW < 0.01; Fig. 3*A*), although the relationship was relatively weak ($R^2 = 0.12$). However, complementarity was strongly related to PD, which was selected as the best predictor ($\beta_{PD} = 0.11$; 95% CI, 0.08–0.15; AW = 0.87; $R^2 = 0.50$; Fig. 3*B*). The plots with the greatest PD resulted in the greatest complementarity, whereas plots with close relatives generally resulted in a negligible or negative complementarity effect (Fig. 3*B*). A negative complementarity effect between close relatives might result from negative interactions that reduce growth in one or both antagonists—such as chemical or physical interference competition (22), or perhaps increased pathogen sharing among close relatives—which has been shown to influence ecosystem function (25).

There was no relationship between either richness or PD and the magnitude of the selection effect (*SI Results*). Instead, the selection effect was related to measures accounting for the abundances of species, including both phylogenetic metrics—namely a measure of the relative skew of abundances across internal nodes in the phylogeny [imbalance of abundances among clades (IAC), $\beta_{IAC} = 0.61$; 95% CI, 0.34–0.88; $R^2 = 0.35$; AW = 0.977; Fig. 3*C*]—and taxonomic diversity (Shannon index, H′: $\beta_H = -37.0$; 95%

CI, −62.96 to −11.12; $R^2 = 0.17$; AW = 0.008; Simpson index: $\beta_{SImp} = -58.07$; 95% CI, −97.18 to −19.18; $R^2 = 0.18$; AW = 0.011). IAC measures the deviation of abundances at internal nodes from a null distribution, where abundances are proportional to the number of descending lineages (26). Thus, high IAC values correspond to an imbalance in abundances in which individual species or clades have disproportionately high abundance. The fact that IAC is a better explanation for the selection effect than either the Shannon or Simpson (nonphylogenetic) index indicates it is more efficacious to include information about the abundances of close relatives than only about individual species.

The sum of the complementarity and the selection effect is the "biodiversity effect" (ΔY), which is the difference between observed and expected polyculture biomass (22). Again, PD was the best predictor of ΔY [$\beta_{PD} = 0.14$; 95% CI, 0.07–0.21; $R^2 = 0.31$; Akaike information criterion (AIC) = 394.26; Fig. 3*D*], but this was only marginally better than IAC ($\beta_{IAC} = 0.84$; 95% CI, 0.43–1.26; $R^2 = 0.30$; AIC = 395.28). Given that these two diversity measures predict different aspects of the biodiversity effect (complementarity vs. selection effect), I looked at a model that included both these variables. PD and IAC were not correlated to each other ($r = 0.01$). Compared with the single predictor models, the model with both PD and IAC as main effects
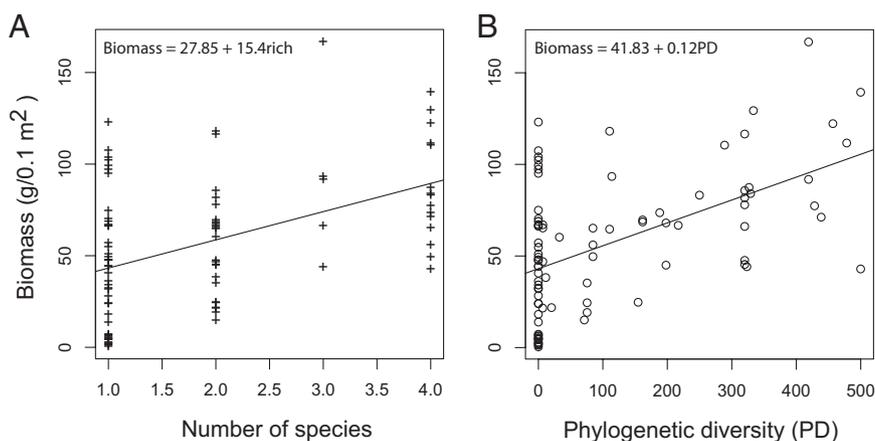

Fig. 2. The relationship between plot biomass production and species richness (*A*) and PD (*B*). Lines are estimated from linear regressions, with models shown in the upper left of each plot.
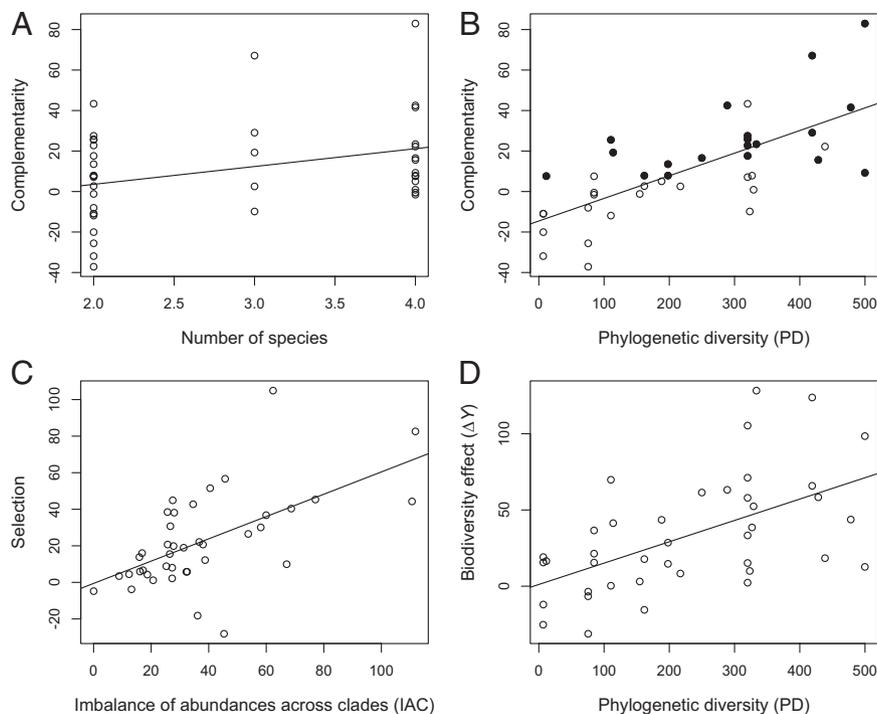
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Fig. 3. The complementarity effect was significantly related to several diversity measures, including richness (*A*) but was explained best by PD (*B*; ●, plots exhibiting transgressive overyielding). The selection effect was predicted best by a phylogenetic measure that quantified the imbalance of abundances across the nodes in the phylogeny (IAC) (*C*). The single best predictor of the overall biodiversity effect was PD (*D*).

was a substantially better predictor of ΔY (AW = 0.99). A model including a PD–IAC interaction was not a significant improvement (AIC$_{main\ effects}$ = 375.95 vs. AIC$_{interaction}$ = 375.85). Thus, the magnitude of the productivity increase in polycultures is explained, to a large degree (R^2 = 0.59), by the combination of two independent phylogenetic measures: the total evolutionary history (PD), which underpins observed complementarity, and a measure of the imbalance in relative abundances across the phylogeny (IAC), which predicts selection effects.

I am using the statistical measure of complementarity as a potential measure of niche differences. Certainly, the measure of complementarity used here can reflect stabilizing niche differences, but not necessarily so (14, 27). Species combinations may show statistical complementarity even when they access the same resources or when the community has not reached equilibrium (27). Thus, transgressive overyielding (when polycultures are more productive than the best performing monoculture of the constituent species) supplies more stringent evidence of niche complementarity (27). Almost half the polycultures (48%) showed transgressive overyielding, and these plots also showed higher complementarity (mean complementarity effect, 26.49 with transgressive overyielding and −3.35 for plots not exhibiting overyielding, and a difference of 29.84; 95% CI, 17.63–42.05; Fig. 3*B*).

The oft-observed relationship between species richness and biomass production has been profoundly important for understanding the potential consequences of species extinction. However, mechanistic explanations largely have been lacking and instead depend on an increased probability of including complementary species in diverse assemblages. The results of many of these biodiversity–ecosystem function experiments have included high variance in biomass production among plots with the same numbers of species. Taken together, this previous work has not been able to provide a method for a priori prediction about which species combinations should result in the highest biomass production. Recent work has shown that phylogenetic measures explain biomass variation better (12, 18). Here, I show that by using

evolutionary relationships, one can predict which species combinations should maximize complementarity and thus polyculture biomass production.

It is important to note that phylogenetic relatedness is not an ecological mechanism in itself. Using a measure such as PD necessarily includes several assumptions about the mode and tempo of ecological differentiation. The basic assumption is that phylogenetic distances are linearly related to ecological differences, which is unrealistic, and future work must develop realistic models relating evolutionary time to ecological differences (28, 29). Furthermore, traits ultimately must explain these ecological differences, yet analyses of plant traits have not been able to provide a more powerful explanation of biomass production (16, 17). It might be that the correct traits, or combinations of traits, have not been measured adequately. Alternatively, it might be that the links between traits and ecological differences are sufficiently complex or idiosyncratic, that an integrated measure such as PD performs better. Using phylogenetic distances as an ecological predictor assumes that species have differentiated from one another (proportional to time) but is ambivalent about which trait(s) have evolved or even whether it is the same trait evolving across different clades.

Phylogenetic relationships are an efficacious predictor of ecosystem function and shed light on why previous experiments have found a positive relationship between productivity and species richness. By using phylogenetic relationships, we can predict which species combinations should be the most productive. These findings support calls to protect evolutionarily distinct species (30, 31) and are in line with recent moves toward conservation goals that include the maintenance of ecosystem function. Conservation needs to consider multiple priorities and species, and evolutionary distinctiveness should be an important part setting conservation goals.

Materials and Methods

This experiment was conducted in a formerly grazed old field at the University of Toronto's Koffler Scientific Reserve, located near King City, Canada (44°02′ N, 79°31′ W). In both fall 2009 and early spring 2010, a

30×30-m field was plowed and disked. Within this field, 100 (10 rows of 10) 2×2-m plots were evenly spaced, separated by 1 m.

Species and Experimental Treatments. In May 2010, the plots were seeded with 1, 2, or 4 plant species from a pool of 17 species, including *Andropogon gerardii* Vitman (Poaceae), *Schizachyrium scoparium* (Michx.) Nash (Poaceae), *Elymus canadensis* L. (Poaceae), *Elymus trachycaulus* (Link) Gould ex Shinners (Poaceae), *Asclepias tuberosa* L. (Asclepiadaceae), *Doellingeria umbellata* (Mill.) Nees (Asteraceae), *Liatris cylindracea* Michx. (Asteraceae), *Rudbeckia hirta* L. (Asteraceae), *Solidago nemoralis* Aiton (Asteraceae), *Oligoneuron album* (Nutt.) G. L. Nesom (Asteraceae), *Desmodium canadense* (L.) (Fabaceae), *Lespedeza capitata* Michx. (Fabaceae), *Monarda fistulosa* L. (Lamiaceae), *Pycnanthemum tenuifolium* Schrad. (Lamiaceae), *Pycnanthemum virginianum* (L.) T. Dur & B. D. Jacks. ex B. L. Rob. & Fernald (Lamiaceae), *Penstemon digitalis* Nutt. ex Sims (Scrophulariaceae), and *Penstemon hirsutus* (L.) Willd. (Scrophulariaceae). These species are strongly associated with tall grass prairie in Ontario and currently do not occur on the reserve in high abundance. The rationale for their use was to establish a "naïve" community, in which, with a priori knowledge, each species has an equal chance of encountering beneficial or negative interactions (e.g., soil pathogens, herbivores, pollinators). Thus, specific species should not have an advantage because of their recent history at the site. Seeds for these species were obtained from Pterophylla Native Plants and Seeds. Each plot was seeded with 3,000–4,000 seeds (which approximates natural seed fall for 4 m^2), evenly divided among the constituent species. After seeding, the soil and seed were compacted using a lawn roller.

After seeding, the plots did not receive any rainfall for 15 d, and I installed a sprinkler system with a gas-powered pump to pull water from four cattle tanks to keep the seeds moist for germination. Also, to suppress weed growth between plots, the interplot space was covered with landscape cloth and pea gravel. Plots were consistently weeded, removing all species not seeded in a plot.

Four species did not germinate in the plots (*D. umbellata*, *L. cylindracea*, *O. album*, and *P. digitalis*). In late fall 2010, *L. cylindracea* and *O. album* were replaced with *Solidago altissima* L., *D. umbellata* was replaced with *Euthamia graminifolia* (L.) Nutt., and *P. digitalis* was reseeded. All new seedings were done at the same seeding densities as the species they were replacing. Unfortunately, neither *E. graminifolia* nor the *P. digitalis* reseeding resulted in sufficient germination. Thus, these two species were dropped from the experiment, and several of the four-species plots became three-species treatments and several of the two-species plots became monocultures.

All species monocultures were replicated three times. The two- and four-species treatments were fully crossed with three PD treatments: (*i*) small phylogenetic distances, in which plots comprised closely related species; (*ii*) large phylogenetic distances, in which plot members were distantly related; and (*iii*) medium phylogenetic distances, in which plots contained moderately distantly related species or a mixture of close and distant relatives (Fig. 1). Because there were fewer possible combinations for the small and large phylogenetic distance treatments, these were replicated seven times each for both the two- and four-species plots. The medium treatments were replicated nine times for both two- and four-species plots.

Sampling. In August 2012, 0.1×1-m quadrats were placed in each plot, 0.25 m away from any plot edge. All above-ground biomass was removed, with stems clipped just above the soil surface. Biomass was sorted into constituent species as well as litter (i.e., dead or dying material not connected to a living stem). All samples were dried in a VWR drying oven (VWR International) at 50 °C for 2 d, then weighed using a Mettler Toledo ML Series precision balance.

Phylogeny. For each species used in this experiment, I queried GenBank (32) for five commonly sequenced genes: *matK*, *rbcl*, *ITS1*, *ITS2*, and *5.8s* (Table S1).

All the species, bar one (*S. altissima*), had at least one gene represented in GenBank; for the missing species, I used gene sequences from a congeneric relative not included in these experiments (*Solidago rigida*). I also included two representatives of early diverging angiosperm lineages—*Amborella trichopoda* and *Magnolia grandiflora*—as outgroup species. Sequences were aligned using MUSCLE (33), and best-fit maximum likelihood (ML) models of nucleotide substitution for each gene were selected using the Akaike information criterion, as implemented in Modeltest (34, 35).

The ML phylogeny using the PhyML algorithm with a BIONJ starting tree (36, 37) was used to estimate the phylogeny. Nodal support was estimated using approximate likelihood-ratio test (aLRT) scores, which have been shown to correlate with ML bootstrap scores but require much less computational time (37). I then used a semiparametric rate-smoothing method (38) to transform the phylogeny to an ultrametric tree using the *chronopl* function in the R package *ape* (39). I iterated this function across a suite of rate-smoothing parameters and found that the parameter value that maximized the likelihood was 1. I then time calibrated the tree by using the *chronogram* function, with the scale set to 160 My—a conservative estimate for the monocot and dicot split (40, 41). The time-calibrated ultrametric ML tree is shown in Fig. 1.

Statistical Analyses. The ability of different measures of diversity to explain variation in biomass was compared. These measures of diversity are listed in Table S2. The reason to compare such measures is that different phylogenetic measures are sensitive to different aspects of topology (e.g., imbalance in diversification rates vs. length of terminal branches) (26). Moreover, it is important to compare taxonomic diversity measures that include abundance (i.e., Shannon and Simpson indices) with abundance-weighted phylogenetic measures. I compared linear models of biomass against each of the diversity measures using Akaike weights (42). Although all model comparisons are not shown in the main article, they are shown in Tables S3–S6.

I quantified complementarity and selection effects according to Loreau and Hector (22). Complementarity is defined as the average difference between the observed relative yield of species in polyculture and the expected yield based on biomass production in monoculture. The selection effect measures the covariance between the relative yield in polyculture and monoculture productivity (i.e., whether species that exhibit high productivity in monoculture also have high relative yield in polycultures). The combination of complementarity and selection is the biodiversity effect, or the amount of biomass produced in polycultures above the expectation from the monocultures. To determine whether complementarity, selection, or biodiversity effects were related to diversity, I compared linear models, with the diversity measures as predictors, comparing them using Akaike weights.

Finally, I assessed transgressive overyielding using the log response ratio (3), $LR = \ln(y_i/y_{m,i})$, where y_i is the biomass of polyculture i and $y_{m,i}$ is the mean monoculture biomass of the most productive species in polyculture i. Thus, a value of 0 means that the polyculture is as productive as the maximum-performing species. Positive values result from transgressive overyielding.

ECOLOGY

1. Tilman D, et al. (2001) Diversity and productivity in a long-term grassland experiment. *Science* 294(5543):843–845.
2. Tilman D, Wedin D, Knops J (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 379:718–720.
3. Cardinale BJ, et al. (2006) Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature* 443(7114):989–992.
4. Cardinale BJ, et al. (2007) Impacts of plant diversity on biomass production increase through time due to complimentary resource use: A meta-analysis of 45 experiments. *Proc Natl Acad Sci USA* 104(46):18123–18128.
5. Balvanera P, et al. (2006) Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecol Lett* 9(10):1146–1156.
6. Zavaleta ES, Pasari JR, Hulvey KB, Tilman GD (2010) Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity. *Proc Natl Acad Sci USA* 107(4):1443–1446.
7. Gamfeldt L, Hillebrand H, Jonsson PR (2008) Multiple functions increase the importance of biodiversity for overall ecosystem functioning. *Ecology* 89(5):1223–1231.
8. Srivastava DS, Vellend M (2005) Biodiversity-ecosystem function research: Is it relevant to conservation? *Annu Rev Ecol Evol Syst* 36:267–294.
9. Duffy JE (2003) Biodiversity loss, trophic skew and ecosystem functioning. *Ecol Lett* 6(8):680–687.
10. Tilman D (1996) Biodiversity: Population versus ecosystem stability. *Ecology* 77(2):350–363.
11. Tilman D, Reich PB, Knops JMH (2006) Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature* 441(7093):629–632.
12. Cadotte MW, Dinnage R, Tilman D (2012) Phylogenetic diversity promotes ecosystem stability. *Ecology* 93:s223–s233.
13. Zavaleta ES, Hulvey KB (2007) Realistic variation in species composition affects grassland production, resource use and invasion resistance. *Plant Ecol* 188(1):39–51.
14. Carroll IT, Cardinale BJ, Nisbet RM (2011) Niche and fitness differences relate the maintenance of diversity to ecosystem function. *Ecology* 92(5):1157–1165.
15. Connolly J, et al. (2011) Phylogenetically diverse grasslands are associated with pairwise interspecific processes that increase biomass. *Ecology* 92(7):1385–1392.

16. Cadotte MW, Cavender-Bares J, Tilman D, Oakley TH (2009) Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. *PLoS One* 4(5):e5695.
17. Flynn DFB, Mirotchnick N, Jain M, Palmer MI, Naeem S (2011) Functional and phylogenetic diversity as predictors of biodiversity—ecosystem-function relationships. *Ecology* 92(8):1573–1581.
18. Dinnage R, Cadotte MW, Haddad NM, Crutsinger GM, Tilman D (2012) Diversity of plant evolutionary lineages promotes arthropod diversity. *Ecol Lett* 15(11):1308–1317.
19. Cadotte MW, Cardinale BJ, Oakley TH (2008) Evolutionary history and the effect of biodiversity on plant productivity. *Proc Natl Acad Sci USA* 105(44):17012–17017.
20. Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science* 316(5832):1746–1748.
21. Spehn EM, et al. (2005) Ecosystem effects of biodiversity manipulations in European grasslands. *Ecol Monogr* 75(1):37–63.
22. Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. *Nature* 412(6842):72–76.
23. Kirwan L, et al. (2009) Diversity-interaction modeling: Estimating contributions of species identities and interactions to ecosystem function. *Ecology* 90(8):2032–2038.
24. Faith DP (1992) Conservation evaluation and phylogenetic diversity. *Biol Conserv* 61(1):1–10.
25. Petermann JS, Fergus AJF, Turnbull LA, Schmid B (2008) Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology* 89(9): 2399–2406.
26. Cadotte MW, et al. (2010) Phylogenetic diversity metrics for ecological communities: Integrating species richness, abundance and evolutionary history. *Ecol Lett* 13(1): 96–105.
27. Turnbull LA, Levine JM, Loreau M, Hector A (2013) Coexistence, niches and biodiversity effects on ecosystem functioning. *Ecol Lett*, 10.1111/ele.12056.
28. Gravel D, et al. (2011) Experimental niche evolution alters the strength of the diversity–productivity relationship. *Nature* 469(7328):89–92.
29. Srivastava DS, Cadotte MW, MacDonald AAM, Marushia RG, Mirotchnick N (2012) Phylogenetic diversity and the functioning of ecosystems. *Ecol Lett* 15(7):637–648.
30. Isaac NJB, Turvey ST, Collen B, Waterman C, Baillie JEM (2007) Mammals on the EDGE: Conservation priorities based on threat and phylogeny. *PLoS One* 2(3):e296.
31. Vane-Wright RI, Humphries CJ, Williams PH (1991) What to protect—systematics and the agony of choice. *Biol Conserv* 55(3):235–254.
32. Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2005) GenBank. *Nucleic Acids Res* 33(Database issue):D34–D38.
33. Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32(5):1792–1797.
34. Posada D, Crandall KA (1998) MODELTEST: Testing the model of DNA substitution. *Bioinformatics* 14(9):817–818.
35. Posada D, Crandall KA (2001) Selecting the best-fit model of nucleotide substitution. *Syst Biol* 50(4):580–601.
36. Anisimova M, Gascuel O (2006) Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Syst Biol* 55(4):539–552.
37. Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 52(5):696–704.
38. Sanderson MJ (2002) Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach. *Mol Biol Evol* 19(1):101–109.
39. Paradis E, Claude J, Strimmer K (2004) APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* 20(2):289–290.
40. Chaw SM, Chang CC, Chen HL, Li WH (2004) Dating the monocot-dicot divergence and the origin of core eudicots using whole chloroplast genomes. *J Mol Evol* 58(4): 424–441.
41. Wikström N, Savolainen V, Chase MW (2001) Evolution of the angiosperms: Calibrating the family tree. *Proc Biol Sci* 268(1482):2211–2220.
42. Johnson JB, Omland KS (2004) Model selection in ecology and evolution. *Trends Ecol Evol* 19(2):101–108.