PNAS
www.pnas.org

Supplementary Information for

High plant diversity and slow assembly of old-growth grasslands.

Ashish N. Nerlekar[1*] and Joseph W. Veldman[1†]

[1]Department of Ecology and Conservation Biology, Texas A&M University, College Station, TX 77843-2258, U.S.A. [*]e-mail: ashishnerlekar@tamu.edu (corresponding author) [†]e-mail: veldman@tamu.edu

* Ashish N. Nerlekar (email: ashishnerlekar@tamu.edu)

This PDF file includes:

Tables S1 to S3
Figures S1 to S9
Legend for Dataset S1

Other supplementary materials for this manuscript include the following:

Dataset S1

www.pnas.org/cgi/doi/10.1073/pnas.1922266117

SUPPLEMENTARY INFORMATION (TABLES)

Table S1. Sensitivity analyses for the global meta-analysis comparing species richness of old-growth and secondary grasslands. Compared to the global meta-analysis (r = 0.5, lnRR = -0.46, Fig. 2), models with plausible covariances that were lower (r = 0.25) and higher (r = 0.75) yielded very similar results (lnRR = -0.46 and -0.45, respectively). An unweighted model (lnRR = -0.48) and a weighted model that excluded the two highest-weighted studies (lnRR = -0.46) confirmed that the global meta-analysis results were not driven by weighting. Columns in the table report: lnRR, the associated confidence intervals (CI), p-values for the heterogeneity test (P), the between-study heterogeneity (I^2), and the number of studies included in the model (n).

Model	Purpose of Sensitivity Test	LnRR	95% CI	P	I^2	n
Weighted mean lnRR, random effects model (r = 0.5)	Global meta-analysis (Fig. 2)	-0.458	-0.637, -0.278	< 0.0001	90%	31
Weighted mean lnRR, random effects model (r = 0.25)	To determine the effect of low plausible covariance estimate on results (*SI Appendix*, Fig. S2)	-0.463	-0.643, -0.284	< 0.0001	91%	31
Weighted mean lnRR, random effects model (r = 0.75)	To determine the effect of high plausible covariance estimate (*SI Appendix*, Fig. S3)	-0.450	-0.629, -0.272	< 0.0001	89%	31
Unweighted mean lnRR	To determine the effect of weighting on the global meta-analysis results (*SI Appendix*, Fig. S4)	-0.484	-0.662, -0.305	< 0.0001	61%	31
Weighted mean lnRR, random effects model (r = 0.5), two highest-weighted studies excluded	To determine whether results were heavily influenced by the two highest-weighted studies (*SI Appendix*, Fig. S5)	-0.461	-0.656, -0.266	< 0.0001	85%	29

Table S2. Tests for bias. We performed three tests to assess publication bias (27, 80, 81), and one test for the influence of sample plot size on lnRR, all of which were negative. The rows describe the tests, associated statistics, test interpretations, and the result of the tests.

Test Description	Test Statistics	Interpretation	Result
Correlation between standardized effect sizes and standard errors (27)	Spearman's rho = -0.176, *P*= 0.343; Kendall's tau = -0.1185, *P*= 0.349	Correlations were not significant	Negative
Cumulative meta-analysis (80) (*SI Appendix*, Fig. S6)	NA	Over time (publication year), effect size became more negative, and converged with global mean lnRR	Negative
Rosenberg's fail safe number (81)	Fail-safe number: 2165	Fail-safe number was greater than the minimum cut-off of 165 (i.e., 5× *n*+10; where *n* = number of studies)	Negative
Relationship between plot size and lnRR (*SI Appendix*, Fig. S7)	Slope: -0.00076, R^2 = 0.0021, *P* = 0.806	Variation in sample area among studies did not influence global mean lnRR	Negative

Table S3. Models to identify potential sources of unexplained variation in Log response ratio. We began by defining a linear mixed effect model of lnRR values, from n = 92 time points, with seven predictor variables as fixed effects, and study sites (n = 31) as random effects. We then used a step-backward selection method based on Akaike Information Criteria (AIC) to identify the best model. We calculated the ΔAIC for each model in relation to the best model. For predictor variables that appeared in models with ΔAIC < 2 (in bold), and were not part of the core hypotheses (i.e., secondary grassland type and latitude, as opposed to secondary grassland age, Fig. 3), we produced supplemental figures to visualize their relationships with lnRR (*SI Appendix*, Fig. S8, S9). Abbreviations are as follows: MAP, Mean annual precipitation; MAT, Mean annual temperature; SG_type, type of secondary grassland; plot_area , size of the sampling unit in each study; Latitude, site location in degrees north or south of the equator; and log_time, base 10 logarithm of secondary grassland age.

Model	**Parameters**	**AIC**	**ΔAIC**	R^2
LnRR~ Continent + Latitude + MAP + MAT + log_time + SG_type+ plot_area	7	85.61	7.38	0.399
LnRR ~ Continent + Latitude + MAP + log_time + SG_type + plot_area	6	83.65	5.42	0.399
LnRR ~ Continent + Latitude + MAP + log_time + SG_type	5	81.71	3.48	0.398
LnRR ~ Continent + Latitude + log_time + SG_type	4	80.34	2.11	0.379
LnRR ~ Latitude + log_time + SG_type	**3**	**78.85**	**0.62**	**0.356**
LnRR ~ Latitude + log_time	**2**	**78.23**	**0**	**0.322**
LnRR ~ log_time	1	85.48	7.25	0.14

SUPPLEMENTARY INFORMATION (FIGURES)

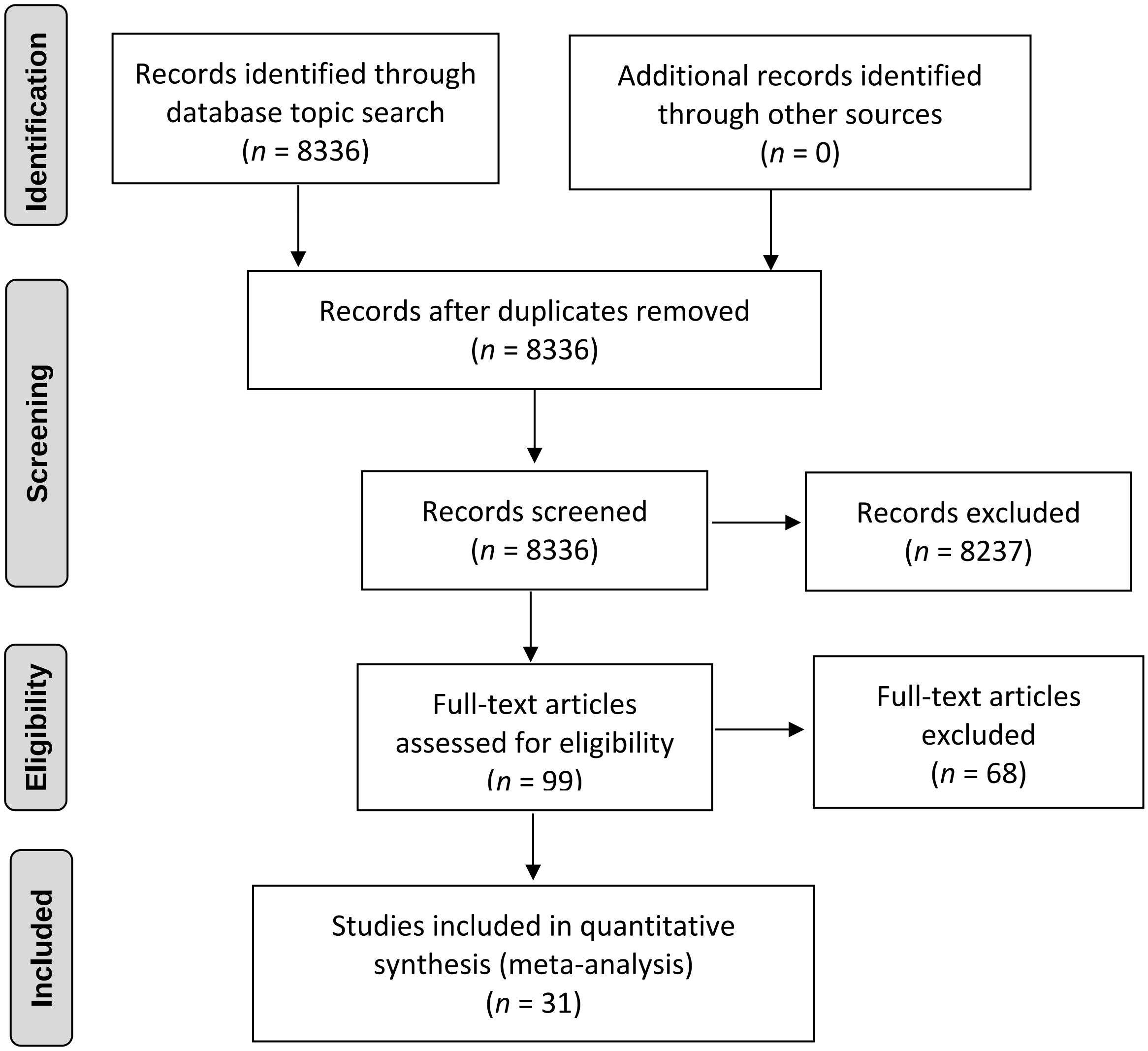

Figure S1. Flowchart of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) for step-wise selection of studies (55). Identification: The Web of Science topic search yielded a total of 8336 articles (we were unable to identify additional records in recent review articles). Screening: We examined the titles of the 8336 articles to eliminate those that were obviously irrelevant (for ambigous titles, we further screened the abstract and methods), which resulted in 99 articles for the final screening: Lastly, we read the full texts of the 99 articles and determined that 31 articles met the eligibility criteria (see Methods) to be included in the analysis.

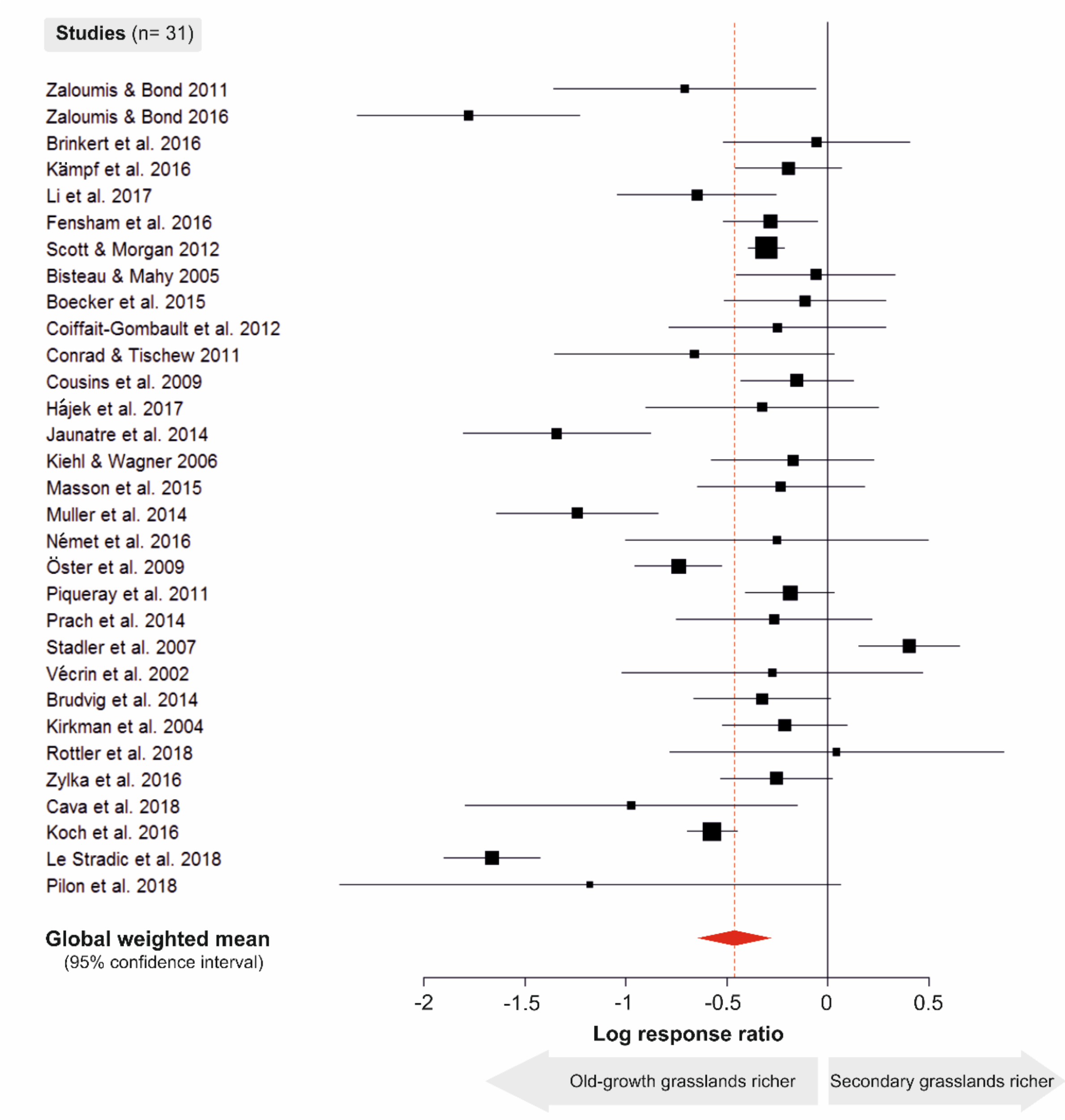

Figure S2. Sensitivity analysis using a plausible covariance of $r = 0.25$. Studies ($n = 31$) are listed alphabetically by continent and author. Boxes and error bars display the natural logarithm of the response ratio (lnRR) and 95% confidence intervals, respectively. Box sizes are proportional to the weight of the study. Log response ratios less than zero indicate that old-growth grasslands are more species-rich than secondary grasslands, whereas values greater than zero indicates secondary grasslands are richer. Displayed as a red diamond and red vertical line, the global weighted mean (lnRR = -0.46, $I^2 = 91\%$, $P < 0.0001$) equates to secondary grasslands supporting 63% of the species richness of old-growth grasslands (*SI Appendix*, Table S1).

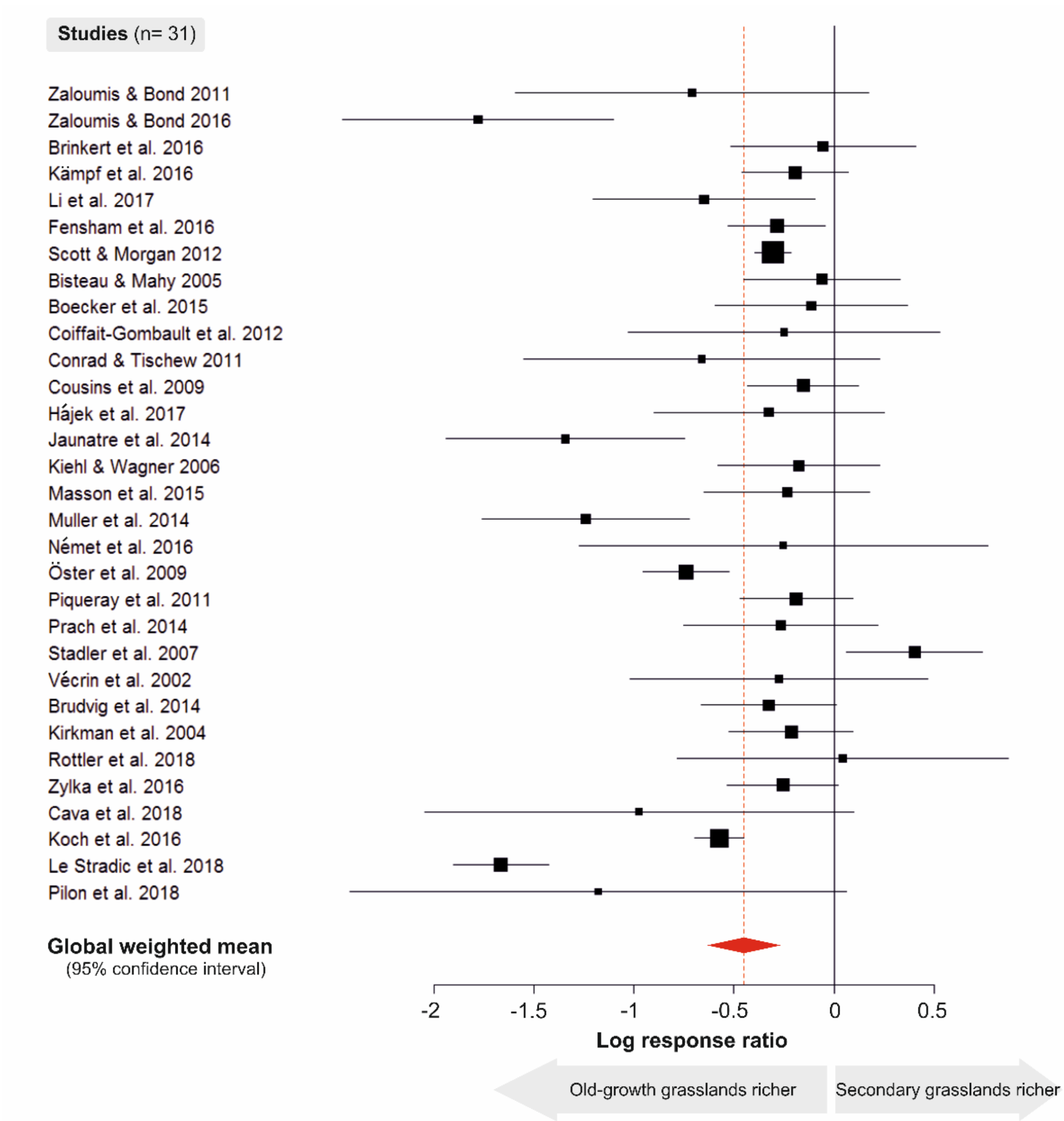

Figure S3. Sensitivity analysis using a plausible covariance of r = 0.75. Studies (n = 31) are listed alphabetically by continent and author. Boxes and error bars display the natural logarithm of the response ratio (lnRR) and 95% confidence intervals, respectively. Box sizes are proportional to the weight of the study. Log response ratios less than zero indicate that old-growth grasslands are more species-rich than secondary grasslands, whereas values greater than zero indicate secondary grasslands are richer. Displayed as a red diamond and red vertical line, the global weighted mean (lnRR = -0.45, I^2 = 89%, P < 0.0001) equates to secondary grasslands supporting 64% of the species richness of old-growth grasslands (*SI Appendix*, Table S1).

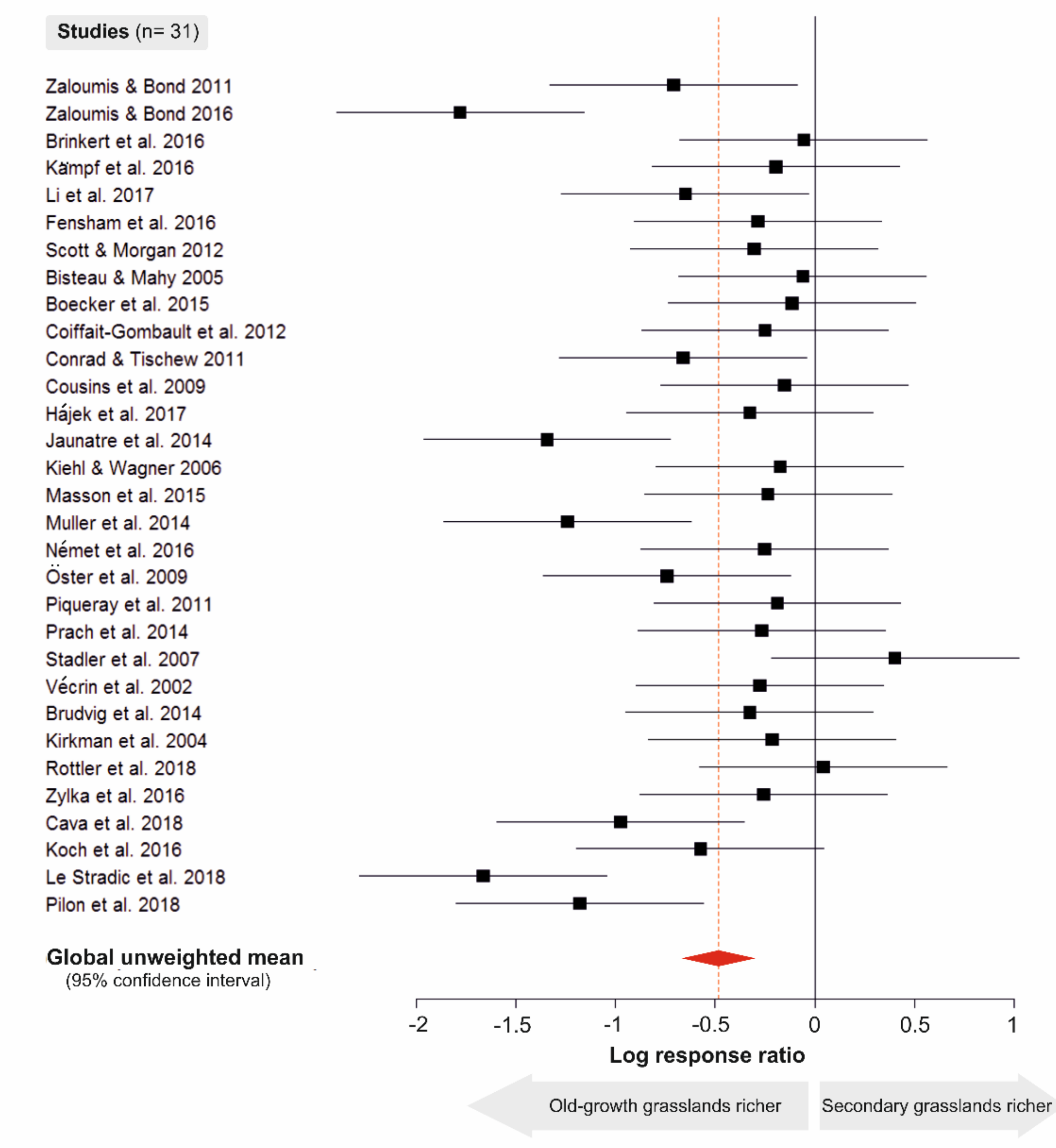

Figure S4. Sensitivity analysis with an unweighted mean lnRR. Studies (*n* = 31 are listed alphabetically by continent and author. Boxes and error bars display the natural logarithm of response ratio (lnRR) and 95% confidence intervals, respectively. Box sizes are proportional to the study weights, which are all equal for this unweighted sensitivity analysis. Log response ratios less than zero indicate that old-growth grasslands are more species-rich than secondary grasslands, whereas values greater than zero indicate secondary grasslands are richer. Displayed as a red diamond and red vertical line, the global unweighted mean (lnRR = -0.48, $I^2 = 61\%$, $P < 0.0001$) equates to secondary grasslands supporting 62% of the species richness of old-growth grasslands (*SI Appendix*, Table S1).

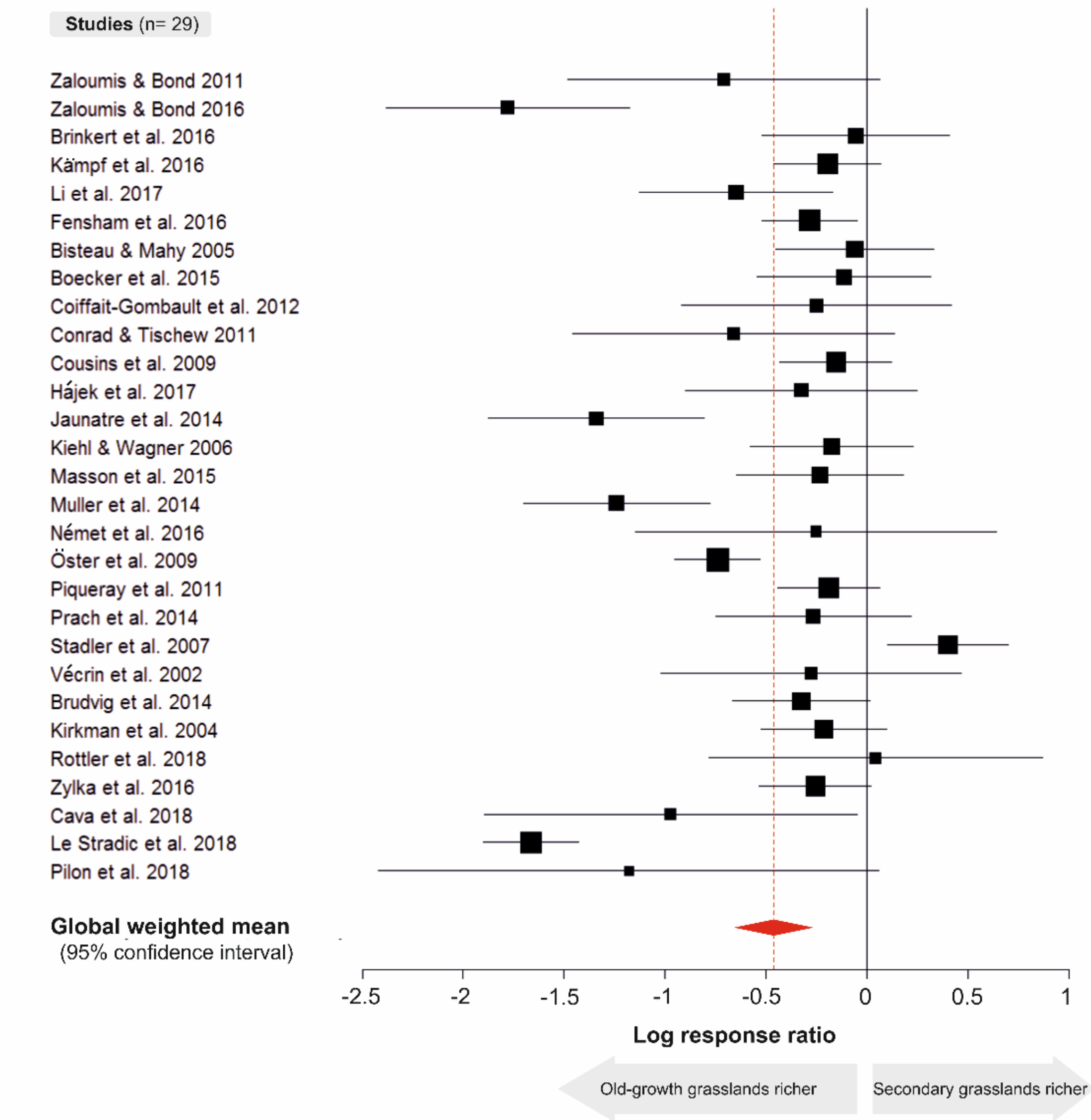

Figure S5. Sensitivity analysis with two highest-weighted studies excluded. Studies (*n* = 29 are listed alphabetically by continent and author. Boxes and error bars display the natural logarithm of the response ratio (lnRR) and 95% confidence intervals, respectively. Box sizes are proportional to the weight of the study. Log response ratios less than zero indicates that old-growth grasslands are more species-rich than secondary grasslands, whereas values greater than zero indicate secondary grasslands are richer. Displayed as a red diamond and red vertical line, the global weighted mean (lnRR = -0.46, I^2 = 85%, $P < 0.0001$) equates to secondary grasslands supporting 63% of the species richness of old-growth grasslands (*SI Appendix*, Table S1)

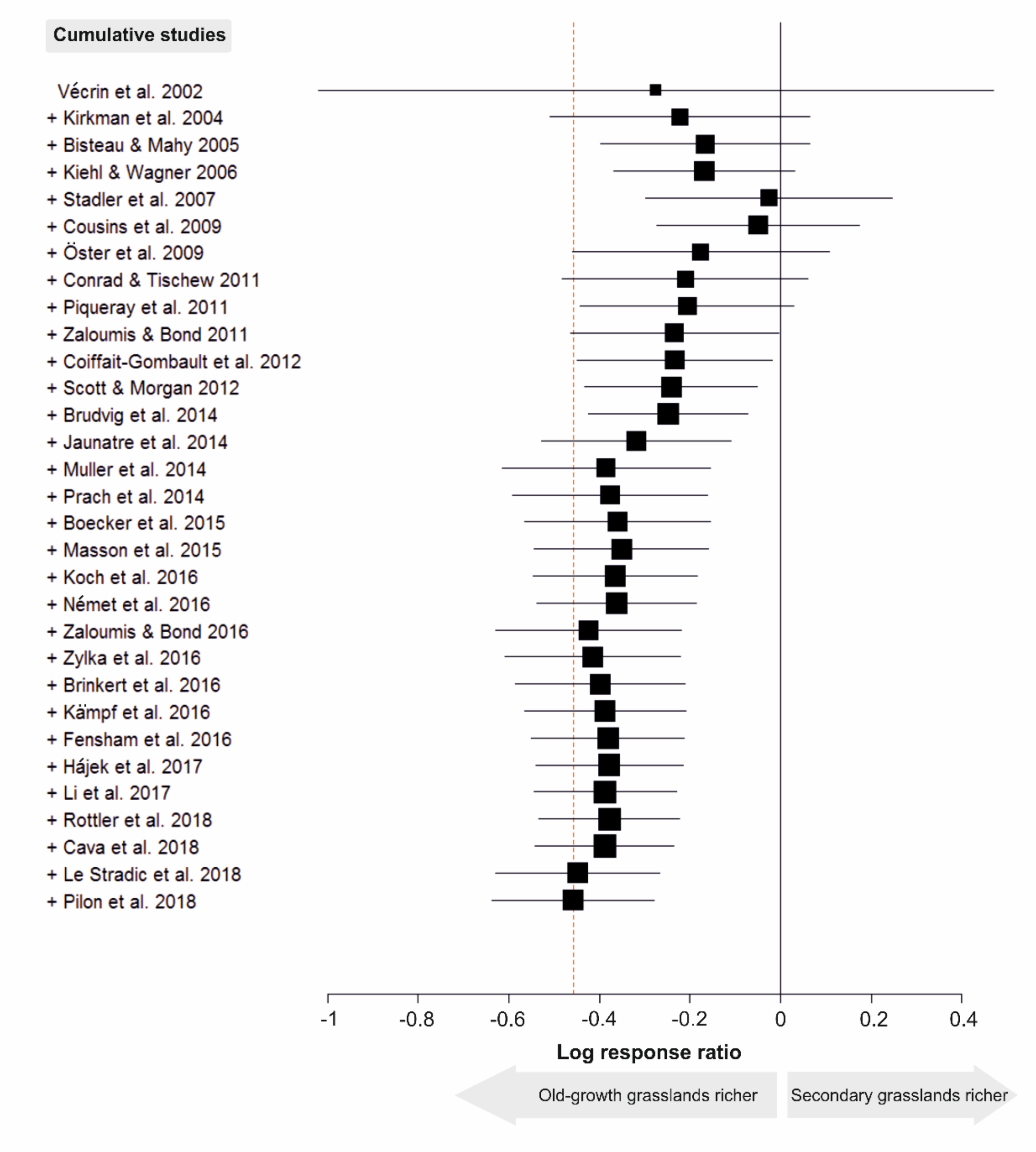

Figure S6. Cumulative meta-analysis to assess publication bias. Studies were sorted by publication year (oldest to most recent) and added one by one to the analysis. With each additional study, the effect size was recalculated using a random effects model. Boxes represent iteratively calculated effect-sizes and the bars represent 95% confidence intervals. The red dashed line represents the global mean (lnRR = -0.46, Fig. 2). Convergence of the iteratively calculated effect sizes with the global mean effect size indicates there is no publication bias.

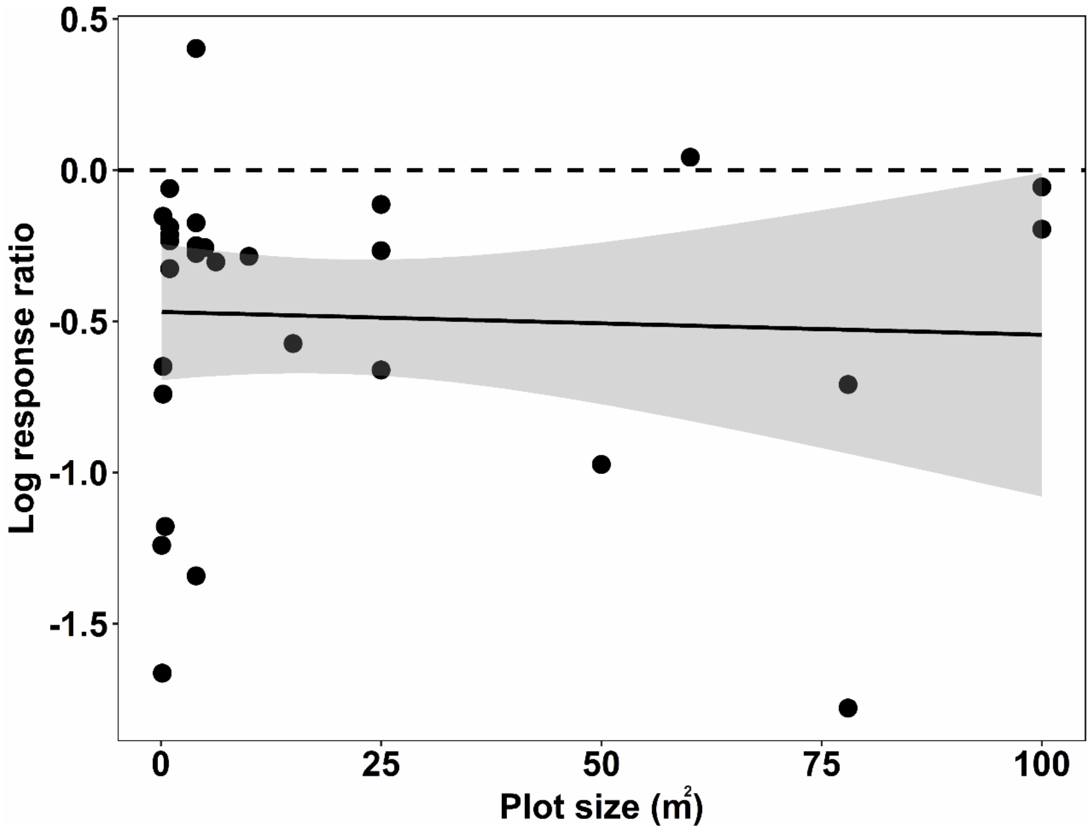

Figure S7. Relationship between plot size and log response ratio (lnRR) of secondary grassland versus old-growth grassland species richness. Because species-area relationships can differ between ecosystems, we sought to determine if variation in sample area between studies influenced lnRR. We extracted information on plot size (which ranged from 0.009 to 100 m^2) from each of the n = 31 studies and conducted a linear regression. The regression equation [lnRR = -0.00076(plot size) – 0.4688, (R^2 = 0.0021, P = 0.806)] is displayed as a solid black line; grey shading indicates the 95% confidence interval. Given that the slope is non-significant and the y-intercept (lnRR = 0.47) is very close to the global weighted mean estimates (i.e., lnRR = 0.46, Fig. 2), we conclude that variation in plot size had no influence on overall results (Fig. 2, Fig. 3).

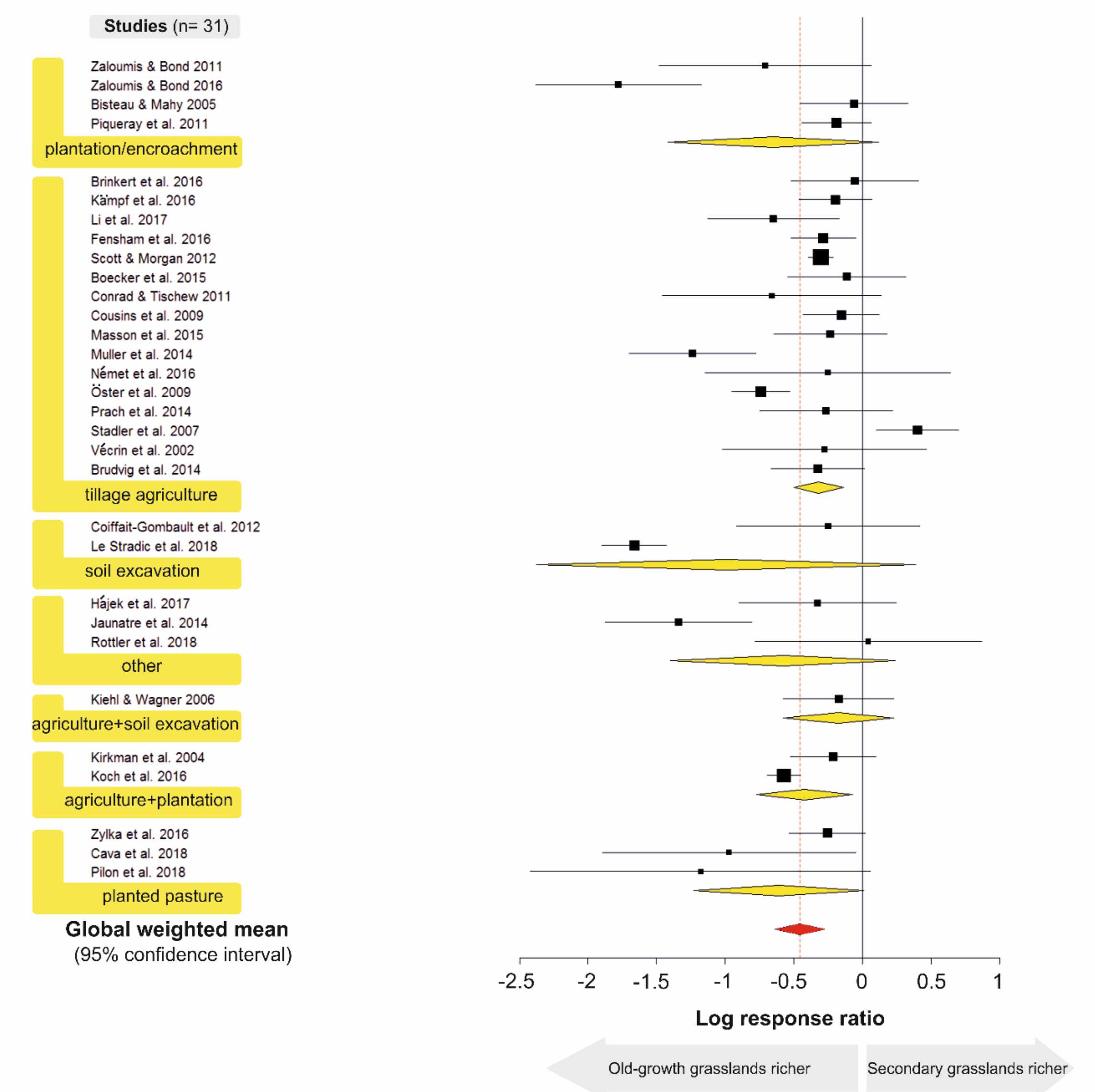

Figure S8. Comparison of old-growth grassland versus secondary grassland species richness based on type of secondary grasslands. Studies (n = 31) are listed by secondary grassland classification. Boxes and error bars display the natural logarithm of response ratio (lnRR) and 95% confidence intervals (CI), respectively. Box sizes are proportional to the weight of the study. Response ratios less than zero indicate that old-growth grasslands are more species rich than secondary grasslands, whereas values greater than zero indicate secondary grasslands are richer. Yellow diamonds represent the weighted subgroup mean and associated 95% CI. The global weighted mean is displayed as a red diamond and red vertical line. ‘Plantation/encroachment’ refers to tree plantations and woody encroachment; ‘agriculture’ refers to tillage agriculture.

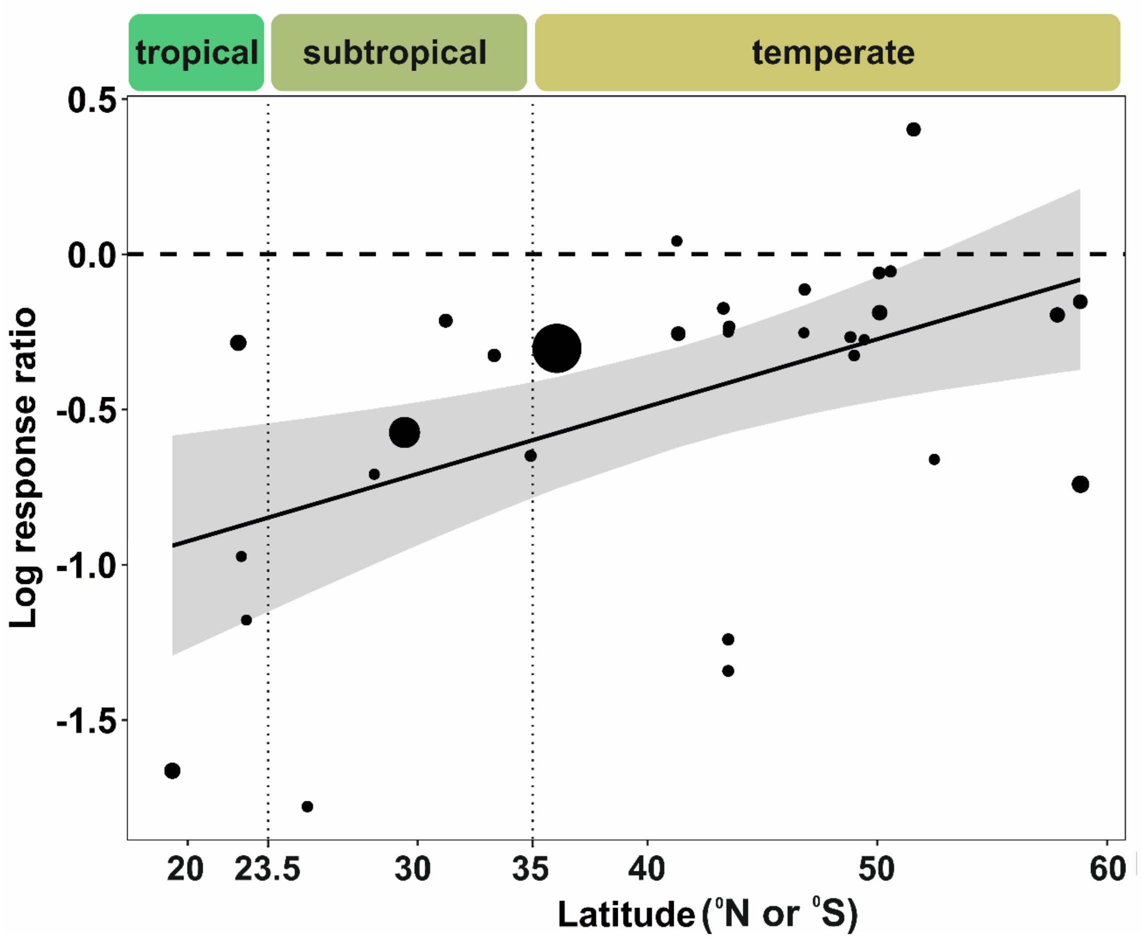

Figure S9. Relationship between the log response ratio (lnRR) of species richness and the absolute latitude of studies. Points represent data from *n* = 31 studies, and are scaled in proportion to their weight (see methods). The regression equation [lnRR = 0.0217(latitude) - 1.358, $R^2 = 0.24$, $P = 0.0026$], is displayed as a solid black line; grey shading indicates the 95% confidence interval. The horizontal dashed line indicates the response ratio at which secondary and old-growth grassland species richness is equal (lnRR = 0). Response ratios less than zero indicate secondary grasslands that have fewer species compared to old-growth grasslands. The labels tropical (*n* = 4), subtropical (*n* = 6), and temperate (*n* = 21) correspond to latitudes of < 23.5°, 23.5-35°, and > 35°, respectively.

Dataset S1 (separate file). Data used for the analyses.