Element interactions limit soil carbon storage

Kees-Jan van Groenigen*†‡, Johan Six*, Bruce A. Hungate§, Marie-Anne de Graaff*†, Nico van Breemen†, and Chris van Kessel*

*Department of Plant Sciences, One Shields Avenue, University of California, Davis, CA 95616; †Laboratory for Soil Science and Geology, Wageningen University, P.O. Box 37, 6700 AA, Wageningen, The Netherlands; and §Department of Biological Sciences and Merriam–Powell Center for Environmental Research, Northern Arizona University, P.O. Box 5640, Flagstaff, AZ 86011

Edited by Christopher B. Field, Carnegie Institution of Washington, Stanford, CA, and approved March 7, 2006 (received for review October 15, 2005)

Rising levels of atmospheric CO_2 are thought to increase C sinks in terrestrial ecosystems. The potential of these sinks to mitigate CO_2 emissions, however, may be constrained by nutrients. By using metaanalysis, we found that elevated CO_2 only causes accumulation of soil C when N is added at rates well above typical atmospheric N inputs. Similarly, elevated CO_2 only enhances N_2 fixation, the major natural process providing soil N input, when other nutrients (e.g., phosphorus, molybdenum, and potassium) are added. Hence, soil C sequestration under elevated CO_2 is constrained both directly by N availability and indirectly by nutrients needed to support N_2 fixation.

global climate change | N_2 fixation | soil organic matter

Numerous studies have reported a surge in plant growth after an abrupt rise in atmospheric CO_2 (1, 2). If increased C assimilation by plants is translated into increased soil organic C content, terrestrial ecosystems might help to mitigate rising anthropogenic CO_2 emissions (3). Simulation models project a wide range of responses of soil C sinks to elevated CO_2. Some models suggest that low nutrient availability will preclude soil C storage (4, 5), whereas others maintain that soil C can accumulate even when nutrient supplies are low (6). Nitrogen fixation, the main source of natural N input in terrestrial ecosystems (7), has been invoked as a process that can potentially diminish N limitation in nutrient-poor systems. Elevated CO_2 has been found to increase N_2 fixation (8), which could provide additional N to support C accumulation in soil. However, N_2 fixation by plants can be limited by the availability of other nutrients such as molybdenum, phosphorus, and potassium (9).

Until now, empirical evidence to evaluate the effect of nutrient availability on soil C storage under elevated CO_2 has been lacking. The effects of nutrient availability and elevated CO_2 are difficult to discern in individual experiments because of high spatial variability in soil C and nutrients and the large amount of C in the soil relative to input rates (10, 11). A quantitative integration of results across multiple studies can overcome some of these problems.

In the current study, we summarized the effect of atmospheric CO_2 enrichment on soil C by performing a metaanalysis on 80 observations from 41 published and unpublished studies. We also summarized the effect of elevated CO_2 on standing root biomass for these studies by using corresponding data for 56 observations on soil C. We divided the studies into three categories of N availability based on N fertilization rates: (*i*) up to 30 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ of N, comparable to maximum atmospheric N depositions in the United States and most of the European Union (12), (*ii*) between 30 and 150 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ of N, typical of extensive agriculture in the United States (13), and (*iii*) >150 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ of N, typical for intensive agriculture in the European Union (13). Similarly, we also evaluated the potential of N_2 fixation to supply extra soil N input under increased CO_2 concentrations by using 92 observations in 25 published and unpublished studies. We compared studies that received additional non-N nutrients and studies that did not. The databases for soil C, root biomass, N_2 fixation, and the results of the metaanalyses can be found in Data Sets 1–7, which are published as supporting information on the PNAS web site.

Results and Discussion

Elevated CO_2 had no effect on soil C in ecosystems receiving up to 30 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ of N (Fig. 1*a*). Soil C accumulation became apparent with increasing inputs of N. Elevated CO_2 increased soil C by 2.1% per year at N additions between 30 and 150 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ and by 2.9% per year at N additions >150 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$. Whether under natural or planted vegetation, in intact or disturbed soils, or with woody or herbaceous species, elevated CO_2 only increased soil C at N additions ≥30 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ (Data Set 4). These results, spanning an array of experimental conditions and terrestrial ecosystems, provide powerful evidence that additional N is needed if C is to be stored in soil under elevated CO_2.

Why is the soil C response to elevated CO_2 restricted by N availability? Because an increase in plant growth under elevated CO_2 causes N to accumulate in litter and plant biomass, soil N availability is expected to limit plant growth under elevated CO_2 in the long term without the addition of exogenous N (14). By contrast, N fertilization can sustain increases in plant growth and, thus, soil C input under elevated CO_2 (15, 16). Indeed, soil N availability limited plant growth under elevated CO_2 for the studies contributing to our soil C database; the response of root biomass to elevated CO_2 increased with N additions (Fig. 1*b*). Because new C enters mineral soil mainly through the root system, these results suggest that the effect of N availability on soil C responses to elevated CO_2 were caused by differences in soil C input.

When ecosystems under elevated CO_2 are subject to N stress, soil microbes might mobilize N through increased decomposition of native soil organic matter (17–19). This so-called priming effect could explain why even in unfertilized experiments, elevated CO_2 significantly increases plant biomass (Fig. 1*b*). Yet, as priming simultaneously increases soil N availability but reduces the soil C reservoir, its potential to accommodate soil C storage is probably small. In fact, the increase in root biomass under elevated CO_2 did not lead to C storage in receiving <30 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ of N studies (Fig. 1*a*). Thus, although N limitation alone does not always preclude positive plant growth responses to elevated CO_2, the interaction between the C and N cycles in terrestrial ecosystems rapidly constrains the CO_2 effect on soil C contents.

Elevated CO_2 also can promote plant N uptake by growing fine roots and mycorrhizae (20–22). However, the potential for such redistributions of N to accommodate C sequestration is small because the resulting rise in plant growth will further increase readily available C and, therefore, microbial N demand (23). Hence, mechanisms that increase plant N uptake without a net ecosystem gain of N are likely to be constrained by

Conflict of interest statement: No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office.

‡To whom correspondence should be addressed. E-mail: cjvangroenigen@ucdavis.edu.

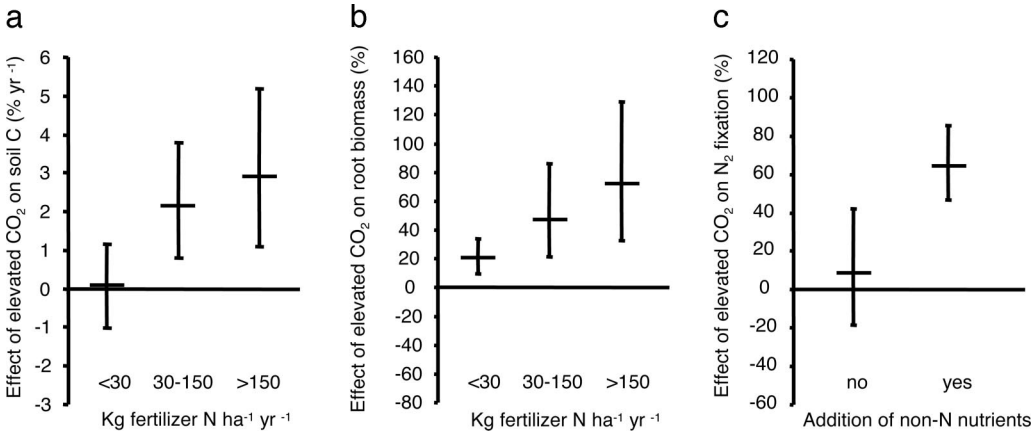

Fig. 1. The effect of elevated CO_2 on soil C contents, root biomass, and N_2 fixation. (*a*) Change in soil C contents as affected by N fertilization. There is a significant difference between N fertilization classes ($P = 0.02$). The values for <30, 30–150, and >150 kg·ha^{-1}·yr^{-1} of N are based on 43, 25, and 12 observations, respectively. (*b*) Change in root biomass as affected by N fertilization. There is a significant difference between N fertilization classes ($P = 0.03$). The values for <30, 30–150, and >150 kg·ha^{-1}·yr^{-1} of N are based on 29, 17, and 10 observations, respectively. (*c*) Change in N_2 fixation as affected by nutrient additions. There is a significant difference between studies that received additional non-N nutrients (43 observations) and studies that did not (49 observations) ($P = 0.02$). All observations are weighted by experiment duration and number of replicates. All error bars represent 95% confidence intervals.

ecological stoichiometry (24) and, therefore, self-limiting (25). Thus, the potential for C storage under elevated CO_2 is highest for ecosystems where plant growth is not limited by N availability. In all other cases, other sources of N are needed to support plant growth and sequestration of soil C.

Nitrogen fixation has been suggested to be one of the other N sources (8). However, our metaanalysis shows that elevated CO_2 had no effect on N_2 fixation under conditions representative of most of Earth's terrestrial ecosystems: With no fertilizer additions, with intact soils, and with naturally occurring plant communities, the response of N_2 fixation to elevated CO_2 was indistinguishable from zero (Data Set 6).

Across the entire data set, elevated CO_2 increased N_2 fixation when other nutrients also were added (Fig. 1*c*). These results suggest that stimulation of N_2 fixation by elevated CO_2 is constrained by the availability of nutrients other than N. In all but one case, non-N nutrient additions included both phosphorus and potassium, which are essential for N_2 fixation (9). Because non-N nutrients were always added in combination (Data Set 3), we cannot test which elements were especially important in releasing N_2 fixation from nutrient limitation. Previous studies have suggested that CO_2 stimulation of N_2 fixation is restricted by the availability of phosphorus (26, 27) and molybdenum (28). Our findings imply that such nutrient constraints are a general feature of N_2 fixation responses to elevated CO_2.

We found no evidence that N fertilization suppressed the response of N_2 fixation to elevated CO_2 (Data Set 6), even though the addition of N fertilizer frequently depresses N_2 fixation (29). Because N addition often occurred in combination with additions of other elements (32 of 45 observations), this result may partly reflect the positive effects of additions of other nutrients overwhelming the negative effects of added N. However, in the subset of observations where we could assess the influence of added N in isolation (in the absence of other nutrient supplements), N fertilization had no effect on the response of N_2 fixation to elevated CO_2 (Data Set 6). Thus, the absence of a response of N_2 fixation to elevated CO_2 without additions of other nutrients was not an artifact caused by N additions.

Elevated CO_2 also increased N_2 fixation in experiments with disturbed soils, an effect comparable in magnitude with that observed by adding non-N nutrients (Data Set 6). Soil disturbance likely operates through its direct effect on nutrient availability, because it generally decreases soil organic matter contents and liberates nutrients (30). Planted communities showed a significantly stronger CO_2 response for N_2 fixation than natural ecosystems. Yet, all studies on natural communities were performed on intact soils, and none of them received non-N nutrient supplements. The CO_2 response between natural and planted communities did not differ on intact and unfertilized soil (Data Set 6). Thus, planting *per se* did not affect the CO_2 response of N_2 fixation. Together, these results suggest that strong responses of N_2 fixation to elevated CO_2 depend on the availability of non-N nutrients.

The effect of CO_2 on N_2 fixation decreased with experiment duration (Data Set 6; $P = 0.001$). One possible mechanism for this decline is identical to that reducing soil N availability in response to elevated CO_2: After an increase in plant growth, micronutrients required for N_2 fixation accumulate in litter and plant biomass (31). The resulting decrease in non-N-nutrient availability limits the response of N_2 fixation to elevated CO_2 or, in some cases, even turns a stimulation into a suppression (28). In addition, light limitation, often absent in short-term experiments, is likely to become more pronounced over time under elevated CO_2 (32). Together, the dependency of N_2 fixation on supporting nutrients, the lack of a response in natural ecosystems, and the decreasing CO_2 response over time strongly imply that the role of N_2 fixation in providing additional N needed for C storage under elevated CO_2 will be small.

Conclusions

Results presented here show that nutrient limitations of plant growth under elevated CO_2 extend to soil C accumulation and N_2 fixation. Our analysis thus provides empirical corroboration for the largely untested hypothesis that large C accumulations only occur with increased inputs or reduced losses of N (4, 5, 24) and broadens it to non-N nutrients. Together, these conceptual, theoretical, and empirical approaches suggest a limited potential for rapid C storage in the terrestrial biosphere after an increase in atmospheric CO_2.

Methods

Data Collection. We extracted results for soil C contents, root biomass, and N_2 fixation from atmospheric CO_2 enrichment studies conducted in the field, in growth chambers, or in glass houses (Data Sets 1–3). We included observations of the effect

of elevated CO_2 that met several criteria. First, the duration of the experimental CO_2 treatment had to be at least 100 days (the approximate length of a growing season in the temperate zone). Second, means and sample sizes had to be available for ambient CO_2 treatments (between 300–400 ppmV) and elevated CO_2 treatments (450–800 ppmV). Estimates of variance were tabulated when available but were not required for inclusion in the analysis. Third, details of experimental conditions needed to be specified. We only included studies that reported experiment duration, soil sampling depth, plant species, and the type of experimental facility. Studies also needed to indicate N fertilization rates. Most studies applied N additions directly to the soil rather than the canopy. This method of N fertilization is bypassing the possibility of foliar N uptake. Consequently, the lower threshold for N effects on soil C storage and root biomass in our metaanalysis is merely an approximation of atmospheric deposition rates.

Finally, studies needed to indicate whether they involved experiments in pots (i.e., any container with dimensions <1 m) or in ecosystems. We made a distinction between studies on intact and disturbed soils. The latter category included all pot studies, studies on reconstructed soils, and studies that applied tillage during CO_2 enrichment.

Because we examined how effects of elevated CO_2 varied with experimental conditions, we included separate observations of elevated CO_2 effects from a single ecosystem under different experimental treatments (e.g., in multifactorial studies). When studies involved more than one level of CO_2 enrichment, we only included results at the level that is approximately twice the ambient CO_2 concentration.

To increase sensitivity for detecting small effects on soil C, we included only surface soil samples, with a maximum depth of 30 cm. When studies reported soil C contents for multiple depths, we included results that best represented the 0- to 10-cm soil layer. Our analysis focuses on mineral soils; thus, we excluded measurements on forest litter layers, marshes, and bogs. Because soil C accumulates slowly over time and the effects of CO_2 enrichment on soil C are often difficult to discern, we only included measurements after the longest exposure period for each study. Soil C contents were all included in the database as a weight percentage. We converted results reported on an area basis to a weight basis by using soil density data whenever available. When soil density data were not reported, we converted results assuming soil bulk density of 1 $g \cdot cm^{-3}$ in ambient CO_2 and elevated CO_2 treatments. We included root biomass data for the whole sampled soil depth. When root biomass data were reported for multiple years, we included data only from the year closest to the year of the corresponding soil C observation.

All forms of biological N_2 fixation (i.e., free-living and symbiotic bacteria and cyanobacteria) were included. N_2 fixation was determined by acetylene reduction, ^{15}N dilution, or N contents of plant tissue when atmospheric N_2 was the only available N source. We explicitly examined the temporal dependence of the N_2 fixation response for multiyear studies by using one estimate per treatment combination per year. Although such measurements are not independent, this approach makes it possible to test whether the responses of N_2 fixation change through time. Eliminating nonindependence by restricting the data set to one estimate per ecosystem-treatment combination did not substantially alter the results (Data Set 7). Although this analysis did reveal a stronger response of N_2 fixation in woody compared with herbaceous vegetation, experiments using woody vegetation were dominated by the use of disturbed soils and the addition of nonnitrogenous fertilizers (19 of 24 observations). In the absence of soil disturbance and non-N nutrient supplements, N_2 fixation did not respond to elevated CO_2 in either woody or herbaceous plants (Data Set 7).

Metaanalysis. Data Sets 1–3 were evaluated by using metaanalysis (33). We used the natural log of the response ratio (r = response at elevated CO_2/response at ambient CO_2) as a metric for the response of N_2 fixation and root biomass to elevated CO_2. These results are reported as the percentage change under elevated CO_2 ($(r - 1) \times 100$). The accumulation of soil C in response to an increase in C input follows a logarithmic pattern over time, yet, in the first 5–10 years, accumulation rates will be approximately linear (34). Because the average duration of CO_2 exposure in the metaanalysis was 3.6 years, we assumed linear changes in soil C over time. Accordingly, we used the natural log of the time-adjusted response ratio $r_t = ((r - 1)/y) + 1$ as a response metric, with y as the length of the study in years. We assumed that most of the soil C input occurs during the growing season. Thus, in order to prevent overestimation of annual changes in the soil C pool in short-term studies (<1 year), we used a minimum of $y = 1$. Results are reported as the percentage change per year under elevated CO_2 ($(r_t - 1) \times 100$).

Well replicated and long-term studies provide more reliable estimates of ecosystem CO_2 responses (10). Thus, we weighted the response metrics by replication and experimental duration by using the function $F_C = (n_a \times n_e)/(n_a + n_e) + (y \times y)/(y + y)$, with n_a and n_e as the number of replicates under ambient and elevated CO_2. Using other weighting functions, such as weighting solely by the number of replicates (35) or weighting all studies equally, did not affect the outcome of our analysis (Data Sets 4–7).

The weighting function conventionally used in metaanalyses, i.e., weighting by the inverse of the pooled variance (by using the largest observed variance for missing estimates) yielded similar results. However, this function calculated weights that differed up to three orders of magnitude in size (Data Sets 1–3). By assigning extreme importance to individual observations, average effect sizes were largely determined by a small number of studies. Thus, we favor the alternative weighting functions because they assigned less extreme weights.

We used a mixed model for analysis of all three data sets, based on the assumption that random variation in CO_2 responses occurred between studies (14) and bootstrapping (4,999 iterations) to calculate 95% confidence intervals around mean effect sizes for categories of studies. P values for differences between categories of studies and for correlation with experiment duration were calculated by using resampling techniques incorporated in METAWIN 2.1 (36).

We thank J. B. West, M. Hoosbeek, S. A. Prior, F. Hagedorn, P. Ambus, P. A. Niklaus, K. Hofmockel, W. Holmes, B. Thomas, F. A. Dijkstra, J. Lichter, R. J. Norby, and W. H. Schlesinger for sharing their data; E. Ainsworth and C. Osenberg for their advice on the statistical procedures; and A. J. Bloom for useful comments on an earlier version of the manuscript. Financial support for this study was provided by the National Science Foundation and the Dutch Ministry of Agriculture, Nature Management, and Fisheries.

1. Schimel, D. S. (1995) *Glob. Change Biol.* **1,** 77–91.
2. Mooney, H. A., Drake, B. G., Luxmoore, R. J., Oechel, W. C. & Pitelka, L. F. (1991) *Bioscience* **41,** 96–104.
3. Gifford, R. M. (1994) *Aust. J. Plant Physiol.* **21,** 1–15.
4. Hungate, B. A., Dukes, J. S., Shaw, R., Luo, Y. & Field, C. B. (2003) *Science* **302,** 1512–1513.
5. Rastetter, E. B., Ågren, G. I. & Shaver, G. R. (1997) *Ecol. Appl.* **7,** 444–460.
6. Cannell, M. G. R. & Thornley, J. H. M. (1998) *Glob. Change Biol.* **4,** 431–442.
7. Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., Asner, G. P., Cleveland, C. C., Green, P. A., Holland, E. A., *et al.* (2004) *Biogeochemistry* **70,** 153–226.
8. Zanetti, S., Hartwig, U. A., Luscher, A., Hebeisen, T., Frehner, M., Fischer, B. U., Hendrey, G. R., Blum, H. & Nosberber, J. (1996) *Plant Physiol.* **112,** 575–583.
9. Vitousek, P. M., Cassman, K., Cleveland, C., Crews, T., Field, C. B., Grimm, N. B., Howarth, R. W., Marino, R., Martinelli, L., Rastetter, E. B., *et al.* (2002) *Biogeochemistry* **57,** 1–45.

10. Hungate, B. A., Jackson, R. B., Field, C. B. & Chapin, F. S., III (1996) *Plant Soil* **187,** 135–145.
11. Schlesinger, W. H. & Lichter, J. (2001) *Nature* **411,** 466–469.
12. Holland, E. A., Brasell, B. H., Sulzman, J. & Lamarque, J. F. (2005) *Ecol. Appl.* **15,** 38–57.
13. *FAO Statistical Yearbook 2004* (2004). (Food Agric. Org. U.N., Rome).
14. Finzi, A. C., Delucia, E. H., Hamilton, J. G., Richter, D. D. & Schlesinger, W. H. (2002) *Oecologia* **132,** 567–578.
15. Curtis, P. S. & Wang, X. Z. (1998) *Oecologia* **113,** 299–313.
16. Oren, R., Ellsworth D. S., Johnsen, K. H., Phillips, N., Ewers, B. E., Maier, C., Schafer, K. V. R., McCarthy, H., Hendrey, G., McNulty, S. G., *et al*. (2001) *Nature* **411,** 469–472.
17. Lekkerkerk, L. J.A., Van de Geijn, S. C. & Van Veen, J. A. (1990) in *Soils and the Greenhouse Effect*, ed. Bouwman, A. (Wiley, New York), pp. 423–429.
18. Zak, D. R., Pregitzer, K. S., Curtis, P. S., Teeri, J. A., Fogel, R. & Randlett D. L. (1993) *Plant Soil* **151,** 105–117.
19. Pendall, E., Del Grosso, S., King, J. Y., LeCain, D. R., Milchunas, D. G., Morgan, J. A., Mosier, A. R., Ojima, D. S., Parton, W. A., Tans, P. P. & White, J. W. C. (2003) *Glob. Biogeochem. Cycles* **17,** GB1046.
20. Zak, D. R., Pregitzer, K. S., King, J. S. & Holmes, W. E. (2000) *New Phytol.* **147,** 201–222.
21. Pregitzer, K. S., Zak, D. R., Curtis, P. S., Kubiske, M. E., Teeri, J. A. & Vogel, C. S. *New Phytol.* **129,** 579–585.
22. Klironomos, J. N., Allen, M. F., Rillig, M. C., Piotrowski, J., Makvandi-Nejad, S., Wolfe, B. E. & Powell J. R. (2005) *Nature* **433,** 621–624.
23. Diaz, S., Grime, J. P., Harris, J. & McPherson, E. (1993) *Nature* **363,** 616–617.
24. Sterner, R. W. & Elser, J. J. (2002) *Ecological Stoichiometry* (Princeton Univ. Press, Princeton).
25. Luo, Y., Su, B., Currie, W. S., Dukes, J. S., Finzi, A., Hartwig, U., Hungate, B., McMurtrie, R. E., Oren, R., Parton, W. J., *et al*. (2004) *Bioscience* **54,** 731–739.
26. Niklaus, P. A., Leadly, P. W., Stocklin, J. & Körner, C. (1998) *Oecologia* **116,** 67–75.
27. Edwards, E. J., McCaffrey, S. & Evans, J. R. (2006) *New Phytol.* **169,** 157–167.
28. Hungate, B. A., Stiling, P. D., Dijkstra, P., Johnson, D. W., Ketterer, M. E., Hymus, G. J., Hinkle, C. R. & Drake, B. G. (2004) *Science* **304,** 1291.
29. Woodward, F. I. (1992) *New Phytol.* **122,** 239–251.
30. Haas, H. J., Evans, C. J. & Miles, E. F. (1957) *U.S. Department of Agriculture Technical Bulletin* **1164,** 1–111.
31. Johnson, D. W., Hungate, B. A., Dijkstra, P., Hymus, G., Hinkle, C. R., Stiling, P. & Drake, B. G. (2003) *Ecol. Appl.* **13,** 1388–1399.
32. Grashoff, C., Dijkstra, P., Nonhebel, S., Schapendonk, A. H. C. M. & van de Geijn, S. C. (1995) *Glob. Change Biol.* **1,** 417–428.
33. Hedges, L. V. & Olkin, I. (1985) *Statistical Methods for Metaanalysis* (Academic, New York).
34. Schlesinger, W. H. (1990) *Nature* **348,** 232–234.
35. Adams, D. C., Gurevitch, J. & Rosenberg, M. S. (1997) *Ecology* **78,** 1277–1283.
36. Rosenberg, M. S., Adams, D. C. & Gurevitch, J. (2000) METAWIN, *Statistical Software for Meta-Analsysis* (Sinauer, Sunderland, MA), Version 2.