Steeply Increasing Growth Differential Between Mixture and Monocultures of Tropical Trees

John J. Ewel[1,4], **Gerardo Celis**[1], and **Laura Schreeg**[2,3]

[1] Department of Biology, University of Florida, Gainesville, FL, 32615, U.S.A.

[2] Department of Ecology and Evolutionary Biology, Brown University, Providence, RI, 02912, U.S.A.

[3] Ecosystem Center, Marine Biological Laboratory, Woods Hole, MA, 02543, U.S.A.

ABSTRACT

Studies of biodiversity and terrestrial ecosystem functioning have concentrated almost exclusively on temperate grasslands. To broaden the reach of biodiversity-functioning research, five fast growing species, comprising three eudicot trees and two congeneric palms (none symbiotic with nitrogen-fixing microorganisms), were grown for 13 yr in a replacement-series mixture and monocultures on a fertile soil in a high-rainfall area of lowland Costa Rica. The mixture accrued more biomass and had greater net productivity than the average, but not the most productive, monoculture. Relative Land Output (a measure of comparative yield) increased steeply. The combined evidence points to an increase in resource partitioning or facilitation among species over time. Spatial partitioning of aboveground space (for light capture) and soil (possibly for retrieval of deep nitrogen), and facilitation of phosphorus availability by one species, are mechanisms that may account for the inferred complementarity. Extending the generalized findings on biodiversity–productivity relationships from well-studied grasslands to tropical forests is warranted. Mixtures of fast growing trees can out-perform the average of their component monocultures, whether the metric is biomass accrual or productivity. The modular growth of long-lived structure enables arborescent species to retain crown space previously captured and may lead to increased spatial partitioning and facilitation of resources over time.

Abstract in Spanish is available in the online version of this article.

DESPITE TODAY'S WEALTH OF DATA SETS, DIVERSITY STUDIES SUFFER FROM A REMARKABLE LACK OF DIVERSITY. Most terrestrial field studies demonstrating richness-productivity responses, and all field studies of time-complementarity responses, have been conducted in temperate-zone communities of herbaceous plants, where aboveground biomass is re-set to zero each winter. What remains untested and unknown is whether relationships between biodiversity and ecosystem functioning hold for systems where growth is perennial and the plants are long-lived. Exploring these questions in tropical forests, with their high species richness, year-round growth potential and large stature of individual trees, is a necessary extension of research on biodiversity and ecosystem functioning.

In recent decades, ecologists have conducted hundreds of studies that address the relationship between biodiversity and ecosystem functioning (reviewed by Hooper *et al.* 2005 and Cardinale *et al.* 2011, see meta-analyses, data assemblies, and syntheses by Balvanera *et al.* 2006, Cardinale *et al.* 2009, Quijas *et al.* 2010). The largest subset of these experiments focused on biomass production as a function of plant species richness, and the bulk of the evidence indicates that biomass production typically (but not universally) increases with the initial number of plant species seeded or grown on a plot (Hector *et al.* 1999, Cardinale *et al.* 2011). Furthermore, the biodiversity–productivity relationship is not static: It can strengthen or weaken as a plant community develops (*e.g.*, Thompson *et al.* 2005, Fargione *et al.* 2007). Time is needed for the most competitive functional group(s) to express dominance (Huston & McBride 2002), for the consequences of plant mortality to manifest themselves, and for complementarity to develop (Cardinale *et al.* 2007, Morin *et al.* 2011). Thus, time is an important axis in describing relationships between richness and production.

Geography and plant habit may constrain the extension of results from temperate grasslands to tropical forests, for there is an important distinction between herbs and trees in their annual partitioning of space, and therefore their ecological combining ability. Perennial herbaceous plants re-sprout each spring and, depending on environmental conditions of the season, one species may fare better this year than last (Grubb *et al.* 1982). The more substantial modular growth of trees, on the other hand, endows them with resistance to interference: A favorable period for growth can result in occupancy of a canopy position that is not quickly relinquished in subsequent years. Shifts do occur over time, as some trees eventually overtop their neighbors (Clark & Clark 1992), or evergreen species compress from below the crown space available to taller deciduous trees (Ewel & Mazzarino 2008). But in the main, space partitioning among trees builds

more on past performance than on annual renewal of competition from ground zero. This is a critical distinction and one that must be accounted for in developing a broader view of biodiversity–ecosystem functioning relationships. Does competitive momentum outweigh the benefits of diversity? Few diversity–ecosystem functioning studies involve trees, and most of them include at least one nitrogen (N)-fixing species, which are well known to facilitate the growth of companion species, at least on N-poor sites during the early years after establishment (Rothe & Binkley 2001).

In addition to the current surge of ecological research on plant diversity and ecosystem functioning, substantial insight on relationships between biodiversity and productivity comes from a long history of work in agronomy and plant population biology (see Trenbath 1974, Harper 1977, Vandermeer 1989). In that research, planting density and spacing arrangement are controlled using replacement-series experiments, in which proportions represented by species change while total initial density is held constant. As a result of replacement-series experiments, we know that the outcome is dependent upon relative abundance (Cousens *et al.* 1987), spatial arrangement (Malézieux *et al.* 2009), timing of arrival (Jolliffe 1988), and plant identity, which in turn reflects a broad suite of functional traits such as architecture (Caldwell 1987, Spehn *et al.* 2000) and physiology (Kitajima & Poorter 2008). In trees, which accumulate structure through perennial growth, these issues should be even more important in determining relationships between diversity and functioning.

The basic metric used to judge the performance of diverse systems is overyielding, which is the ratio of the yield of the mixture to the average monoculture yield of its component species; overyielding occurs when the ratio is > 1, and it is the most typical outcome in replacement-series comparisons of mixtures with monocultures of their components (Jolliffe 1997). A more stringent measure of performance is transgressive overyielding, which is the ratio of yield of the mixture to that of the most productive monoculture. Thus, transgressive overyielding > 1 sets the bar considerably higher and is solid evidence of resource partitioning or facilitation. The converse, however, is not true: the absence of interspecific complementarity cannot be inferred from transgressive overyielding ≤ 1.

Although replacement experiments provide a direct assessment of the performance of species mixtures, they are seldom employed for trees because the plots would consume vast areas and, in most environments, take a long time to yield results. Furthermore, most replacement-series tree experiments are either restricted to two-species combinations (*e.g.*, Brown 1992, Garber & Maguire 2004), or include an N-fixing species (Forrester 2013). Most tropical tree replacement-series experiments have heretofore been inconclusive, either because of complete mortality of some species (Piotto *et al.* 2010), because stand youth and tree mortality resulted in non-significant overyielding (Potvin *et al.* 2011), or because of the chance inclusion of exceptional performers in the mixtures (Erskine *et al.* 2006).

For 13 yr, we tracked survival and growth of five potentially complementary species grown in a single combination and in monocultures of identical initial density. Growth was measured by both accrued biomass (an amount) and by productivity (a rate). The experiment's replacement-series design enabled us to compare the yields and relative land outputs of the mixture and monocultures, and to measure the consequences of tree mortality as the stands developed. By locating the experiment in one of the world's least-constraining environments, we witnessed early onset of competition, fast growth, and the revelations of outcomes in just a decade—a rapid time course for a community of large-stature perennial plants.

METHODS

STUDY site.—The research was conducted at La Selva Biological Station, located on the Atlantic coastal plain of Costa Rica (10°26′N, 83°59′W, 41 masl). Annual rainfall during the 13-yr duration of the experiment ranged from 2.9 to 5.3 m and averaged 4.3 m. A drier season occurs from January through April, but no month has a long-term mean rainfall of <0.1 m. The study site occupies a flat alluvial terrace on a peninsula at the confluence of two rivers: Sarapiquí and Puerto Viejo. When the study was initiated the site was occupied by second growth forest that had colonized an abandoned cacao plantation. That vegetation was felled and broadcast-burned prior to establishment of the experimental plantations.

The study-site soil is very fertile in comparison with most soils of the humid tropical lowlands. It is an allophanic Andisol (Eutric Hapludand) characterized by high cation exchange capacity and amorphous clays that bind organic matter strongly (Ewel *et al.* 2014). Surface soil bulk density is low (~ 0.7) and pH is relatively high (~ 5.5–6.5). Soil conditions were extremely homogeneous across the study site.

SPECIES.—We used five tree species: three eudicots and two arborescent palms. We chose species that represented a range of architectures and phenologies; were known, or assumed, to grow well at the study site; and had readily available seed of known origin. We deliberately excluded trees symbiotic with N-fixing microorganisms. After first mention, eudicot tree species are referred to by genus name. *Hieronyma alchorneoides* Allemão (Phyllanthaceae) is a large-stature, long-lived evergreen tree. Its foliage is dense, and little sunlight penetrates the closed canopies of monospecific plantations of this species. *Cedrela odorata* L. (Meliaceae) is a broad-crowned tree that, in the study-site climate, tends to be deciduous in the dry season. *Cedrela* is subject to debilitating herbivory of its terminal shoots by the larval stage of a moth, *Hypsipyla grandella* Zeller. If growing vigorously it re-sprouts after shoot borer attacks, however, and if it attains a height of about 5 m borer damage declines to almost nothing. *Cordia alliodora* (R & P) Cham. (Boraginaceae) is a second-growth species that has long internodes and a narrow, open crown which allows substantial light penetration to the soil. *Cordia* is not an N-fixer, but it does accrue substantial quantities of N, such that leaves of

individuals growing on the fertile soil at the study site typically had mass-based N concentrations of 3–4 percent.

The two palms (Arecaceae) were congeners. *Euterpe macrospadix* Oerst. produces a single (sometimes a few) main stems attaining heights of 20 m and has pinnate leaves about 3 m long. *Euterpe oleracea* Mart. produces up to 20 stems in each clump and attains heights of 25 m; its pinnate leaves are about 3 m long. The use of congeneric palm species was motivated by initial uncertainty regarding our ability to acquire sufficient seeds of *E. macrospadix* and to successfully produce its seedlings in the nursery. All of the species studied, with the exception of *E. oleracea* which is native to the Amazon floodplain, are native to the local area.

EXPERIMENTAL DESIGN.—The experiment consisted of two replications ($\approx$1376 m^2 each) of a five-species mixture and three replications (1200 m^2 each) of equal-density monocultures of each eudicot species. We grew both the two palm species in two Nelder type I.a. plots (Nelder 1962) used to assess the effects of plant density on growth and survival. Palm seedlings were planted at 11 points along radii spaced at $\pi/8$ radians emanating outward from a central point; plant spacing increased along each radius from 0.25 m to 4.7 m in the first to tenth annulus, respectively. We used individuals growing in the annulus that closely approximated the spacing in monocultures and mixtures to estimate palm growth in monocultures. The Nelder plots were eliminated (due to the risk of pollen release by the alien *E. oleracea*) after 9 yr. All plots—mixtures, monocultures, Nelders—were in close proximity on the same 8 ha study site, all were planted as close as possible to the same date, and all employed uniform nursery stock grown from seeds of known maternity.

Planting density in the mixtures and eudicot monocultures was about three times higher (2887 individuals per hectare; 2.0 m on-center) than would normally be used in a commercial plantation of trees. The hexagonal planting pattern in the mixture was designed to maximize the diversity of neighbors surrounding each individual. Thus, each tree was equidistant (2.0 m) from six individuals, none of which was a congener, and no more than two of which were the same species. Each genus was planted in every row, but the congeneric palms were planted in alternate rows. Thus, the number of each species of palm (*E. macrospadix* or *E. oleracea*) was half that of the number of each eudicot. Each replication contained 100 (range = 98–100) individuals of each species (genus in the case of *Euterpe*).

To compare monocultures and mixtures, it is important that the monocultures express the potential of the species and grow at their maximum rate (*e.g.*, Vandermeer 1989). Therefore, because of their high density and high survival (*ca* 100%), the eudicot monocultures were periodically thinned lightly from below. This involved removal of smaller individuals (subdominants in the lexicon of foresters) whose crowns were encroaching on those of canopy trees; care was taken never to expose the crown of any residual tree on more than one side. Judicious

thinning of high-density monocultures of modular-growth plants reduces intraspecific competition and avoids stand stagnation while ensuring full use of the site's resources. The five-species plots, where intraspecific competition was presumably less intense than the intraspecific competition in monocultures, were never thinned.

MEASUREMENTS AND CALCULATIONS.—Size (heights, diameters, frond counts of palms) and survival of all plants were measured regularly for 13 yr (9 yr for the palms in Nelder plots). The first four measurements were made every ~ 6 mo; after age 2 yr, re-measurements were done annually. Litterfall was captured in 12 (mixtures) or three (monocultures) screen-bottomed traps in each replication of each block. Litter was collected every 14 d, separated by type (leaf, branch, reproductive parts), oven-dried, then weighed.

We calculated biomass from the plant-size measurements using allometric equations developed for these species (Cole & Ewel 2006). There were two exceptions as we estimated aboveground biomass of *E. macrospadix* using the published equation for *E. oleracea*, and calculated root biomass of both species of *Euterpe* from the following relationship based on the largest ramet in a clump:

$$Euterpe\ root\ \text{biomass(kg)} = 0.026\big(d^2 h\big)^{0.759}, \text{where}$$
$$d = \text{diameter[cm]at1.3m}\ and$$
$$h = \text{height(m)}.$$

To develop the allometric equations, >150 individuals of each species that encompassed the full range of ages and sizes included in this study were destructively harvested at the study site. For the first 7 yr, the entire root system of destructively harvested trees was excavated. Thereafter, it became impossible to determine which roots came from which individual, so we changed the root-sampling protocol to a 1.0-m diameter cylinder centered on each tree selected for harvest. The cylinder was excavated to the depth required to recover all roots $\geq$5 mm in diameter. We calculated aboveground net primary productivity (ANPP) as the biomass change during a measurement interval, plus the mass of litter (and any thinned material, on occasions when monocultures were thinned) collected during that interval.

We compared mixtures and monocultures by calculating overyielding (ratio of mixture yield to that of average monoculture) and transgressive overyielding (ratio of mixture yield to that of most productive monoculture) for each year (biomass) or interval between years (ANPP) for years 0–9, after which palm monoculture data were no longer available. We used a single denominator in calculating overyielding for each year because the monoculture data for the two palm species were based on relatively few individuals.

To further assess the relative performance of mixtures and monocultures (measured as accrued biomass and ANPP), we used the Relative Land Output (RLO) index of Jolliffe (1997)

which compares biomass (or ANPP) in a mixture with an equal land area composed of as many monocultures as there are species in the mixture; normally, each monoculture is sized in proportion to the relative abundance of its corresponding species in the mixture: 0.25 for each eudicot, 0.12 for *E. macrospadix*, and 0.13 for *E. oleracea* in our case. For the special case of replacement-series experiments, calculation of RLO reduces to:

$$\text{RLO} = (\Sigma X_{i,\text{mix}})/\Sigma(X_{i,\text{mon}} * p_i)$$

where $X_{i,\text{mix}}$ = biomass (Mg/ha) or ANPP (Mg/ha/yr) of species i in mixture, $X_{i,\text{mon}}$ = biomass (Mg/ha) or ANPP (Mg/ha/yr) of species i in monoculture, and p_i = proportion of individuals of species i in mixture.

Replacement-series designs, deployed primarily for small-stature, short-lived plants, assume complete survival throughout the usual one-season growth cycle. That assumption rarely holds for trees, and our study was no exception. Therefore, we calculated RLO using both the initial proportion of each species in the mixture as well as the actual proportion of each species as the stands developed and individuals of different species died at different rates.

RESULTS

SURVIVORSHIP AND DENSITY.—Survival in mixtures varied considerably among species (Fig. 1A). By the end of the study, 80–90 percent of the initial number of two species, *Hieronyma* and *E. oleracea*, were still alive, while survival of two others, *Cordia* and *E. macrospadix*, was 40–50 percent. In contrast, *Cedrela* survival in the five-species stands was only about 5 percent, a fate attributable to its response following *Hypsipyla* attack.

While the density of plants in the mixture changed in response to natural mortality, the density in the eudicot monocultures changed in response to both natural mortality and light thinning (Fig. 1B). Density was comparable in the mixtures and monocultures for the first 4–5 yr, after which the mixtures had greater stem density than the monocultures; intraspecific competition was presumably less intense in the mixtures than the intraspecific competition in the monocultures. Even at the end of the study, however, all eudicot monocultures were still densely stocked (>700 trees/ha).

GROWTH.—Growth of the species that had good survival was extremely rapid, as anticipated (Table 1). Maximum heights ≥ 26 m and diameters up to 24–38 cm for the three eudicots resulted in high biomass; some individuals of *Hieronyma* and *Cordia* weighed >450 kg. The two palms, which (like all palms) have limited stem thickening, were smaller than the eudicots and therefore accumulated less biomass—up to about 160 kg per individual for *E. oleracea* and a maximum of only ~13 kg per individual for *E. macrospadix*.

Stand-level basal area is a useful measure of performance because it integrates both the diameter increment and surviving
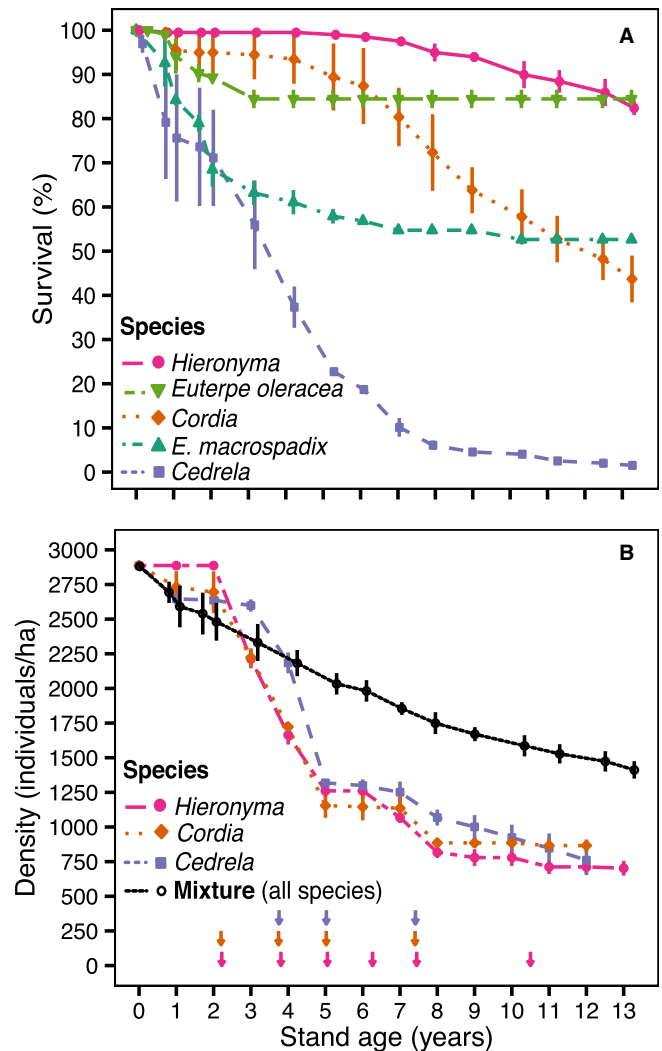

FIGURE 1. Plant survival and stand density. (A) Survival of three eudicot tree species and two congeneric palms grown together. (B) Density of three eudicot trees grown in monocultures (lightly thinned at times indicated by arrows) and mixtures (unthinned) of five species. Values are means ± SE.

number of individuals. Basal area was higher for all species in monoculture than mixture throughout, although never to an extent that reflected the four-fold difference in initial planting density (Figs. 2A and B). The monoculture-mixture difference was especially large for *Cedrela*, which had low survival in the mixture (Fig. 1A). After the first 5 yr, by which time the mixture and the *Hieronyma* monoculture each had accrued about 20 m²/ha of basal area, the mixture outpaced all monocultures, and by age 13 yr had 46 m²/ ha. There is little evidence of a depression in basal area increment due to the occasional thinning of the monocultures (Fig. 2B).

Biomass (including roots) tracked basal area increment reasonably closely, as expected, although the time courses of biomass increment were almost linear, whereas those of basal

TABLE 1. *Sizes of individuals of five species in mixture and in monocultures at stand age 9 yr. Values for the two palms,* E. macrospadix *and* E. oleracea, *in monoculture are based on fewer individuals, sampled in spacing trials. Values are means (standard error), [range]. Biomass includes only the roots in a 1.0-m diameter cylinder centered on the tree.*

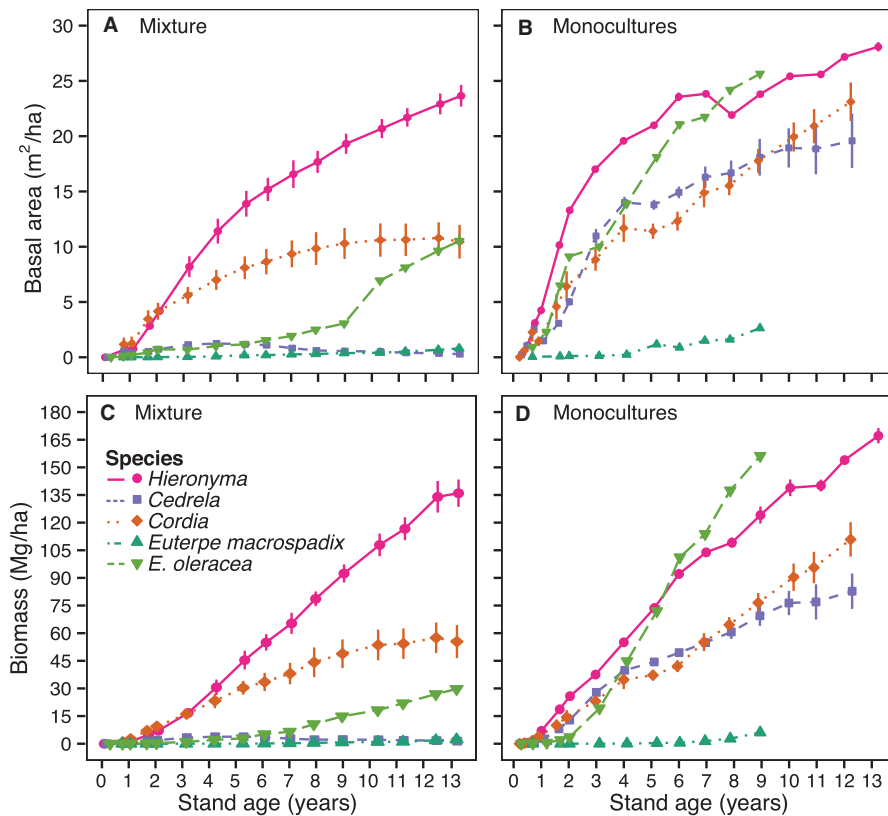| Species	Height (m)		Diameter (cm)		Biomass (kg)	
	Mixture	Monoculture	Mixture	Monoculture	Mixture	Monoculture
Hieronyma alchorneoides	18.6 (0.3)	21.7 (0.2)	18.3 (0.4)	18.7 (0.2)	135.9 (7.0)	148.0 (4.8)
	[5.7–26.7]	[10.8–28.0]	[6.8–31.0]	[9.5–29.0]	[10.8–452.5]	[18.1–454.5]
Cedrela odorata	17.8 (1.2)	15.9 (0.3)	14.2 (1.4)	14.9 (0.3)	70.2 (17.6)	72.6 (4.0)
	[13.0–23.4]	[6.8–28.2]	[9.0–22.2]	[6.8–29.6]	[18.3–188.2]	[10.0–403.5]
Cordia alliodora	20.1 (0.4)	18.2 (0.3)	16.0 (0.5)	15.6 (0.3)	106.4 (7.5)	89.5 (4.5)
	[1.3–33.2]	[7.9–27.2]	[2.9–32.8]	[6.5–31.0]	[1.4–488.2]	[10.2–377.0]
Euterpe macrospadix	3.4 (0.2)	4.2 (1.0)	4.9 (0.1)	6.6 (1.1)	3.8 (0.3)	8.4 (3.4)
	[0.5–5.7]	[2.3–5.9]	[2.1–7.0]	[4.4–7.8]	[0.1–8.3]	[2.0–13.4]
Euterpe oleracea	8.5 (0.3)	10.5 (1.3)	10.8 (0.3)	11.4 (1.1)	47.1 (3.3)	67.9 (18.3)
	[1.9–16.0]	[3.5–16.8]	[3.7–16.7]	[5.3–16.6]	[1.9–162.9]	[4.9–163.9]

FIGURE 2. Basal area and biomass of each species in monoculture and mixture. (A) Basal area in mixture. (B) Basal area in monocultures. (C) Biomass accrual, including roots, in mixture. (D) Biomass accrual, including roots, of monocultures. Biomass for years 8–13 is an underestimate of the true value because roots were sampled only under the 1.0-diameter cylinder directly beneath each tree and not in the spaces between trees. The same scale is used on both panels in each pair, so the totals for the mixtures (off scale) are the sums of the values graphed, *e.g.,* 46 m²/ha and 225 Mg/ha in year 13 for basal area and biomass, respectively. Values on all panels are means ± SE.

area was more hyperbolic (Figs. 2C and D). In mixed-species plots, *Hieronyma* accounted for more biomass than any other species from about age 5 yr onward, and by the end of the study it accounted for more than half of stand biomass. The rate of stand-level biomass accrual in the mixtures was very rapid, averaging >17 Mg/ha/yr, which resulted in a standing crop of ~225 Mg/ha by age 13 yr, compared with the maximum biomass accrual by any monoculture of 160 Mg/ha (by *Hieronyma*).

It is important to note that, from age 8 yr onward, total biomass is underestimated because root biomass was based on a 1.0-m diameter cylinder around each tree; roots in the interstices between trees were not sampled. Nevertheless, we are confident that the relative biomass estimates in monocultures and mixtures are unbiased, based on unpublished data from the monocultures and adjacent three-species plots where roots were sampled more intensively using a combination of the 1.0-m diameter cylinders and 1.0 × 0.73 m inter-tree pits.

Aboveground net primary productivity (ANPP) in the five-species mixture was higher than that of any eudicot monoculture and averaged ~ 28 Mg/ha/yr during the last 10 yr of the study (Fig. 3). The most productive of the eudicot monocultures, *Hieronyma*, exceeded that of the mixture during the first year and was about the same as that of the mixture during years 2–7. Thereafter, the mixture sustained its high ANPP, whereas that of the *Hieronyma* monocultures declined to ~17 Mg/ha/yr through year 13. Monocultures of *Cordia* and *Cedrela* had about half the ANPP of the mixtures. The exotic palm, *E. oleracea*, had exceptionally high ANPP during growth-year 5, but we caution that the ANPP data for palms in monoculture is based on relatively few individuals and data from different Nelder plots were pooled.

The initial density of each species (genus in the case of *Euterpe*) in mixture was only one-fourth its density in monoculture, so if resource pools per individual were the same in both, the expected biomass of any species in mixture would be 25 percent (12.5 percent for each of the palm species) as large in mixture as in monoculture. That was not the case, as illustrated by the differences between observed and expected growth (Fig. 4). Two species, *Hieronyma* and *Cordia*, grew much faster in mixture than in monoculture; two others, *Cedrela* and *E. oleracea*, fared better in monoculture than in mixture; and the fifth species, *E. macrospadix*, grew slowly and at about the same rate in both mixture and monoculture. The enhanced performance of *Hieronyma* and *Cordia* in mixture more than compensated for the losses in growth incurred by the other species.

RELATIVE PERFORMANCE.—Overyielding, in which the yield of the mixture exceeds the mean yield of component monocultures, occurred every year (except yr 1) for both biomass and ANPP (Fig. 5). Transgressive overyielding, in which the yield of the mixture exceeds the yield of the most productive monoculture, occurred very infrequently, and in most years values hovered around 1.0 (Fig. 5).
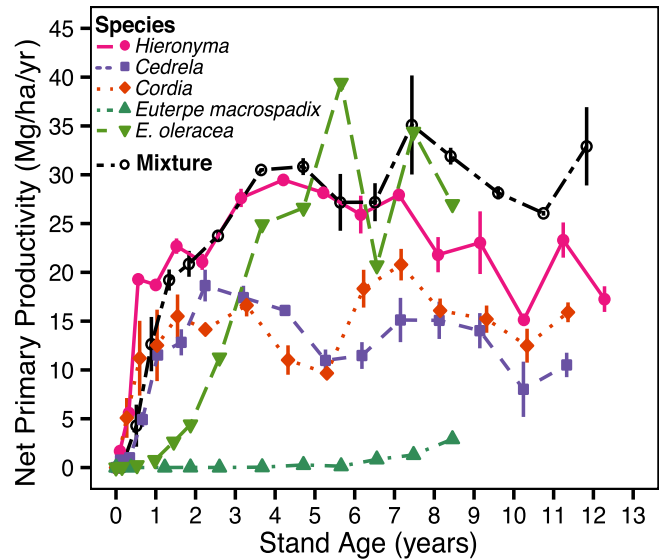

FIGURE 3. Aboveground net primary productivity (ANPP) of the mixture of five species and each component species in monoculture. Values are means ± SE.
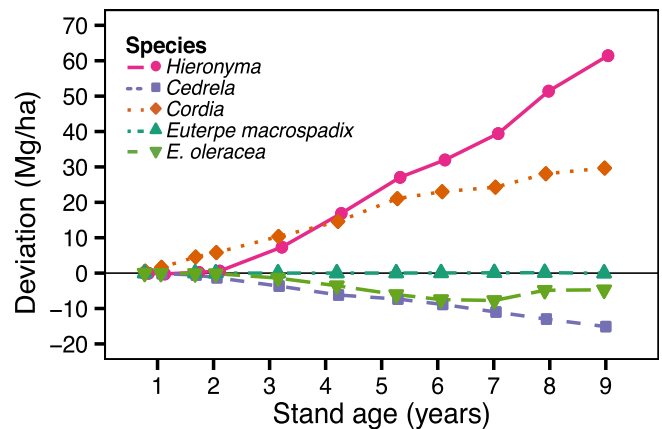

FIGURE 4. Deviation of biomass (observed minus expected) for each species over time. Observed is the value in mixture; expected is the proportion of the mixture initially constituted by that species times its biomass in monoculture.

Four RLO results (Fig. 6) are especially noteworthy and constitute our most important findings: (1) the mixtures accrued more biomass and had higher ANPP than the area-weighted monocultures throughout the study; (2) RLO increased over the course of 9 yr whether measured as biomass or ANPP despite the fact that productivity is a rate function and typically levels or oscillates around an asymptote shortly after crown closure; and when the actual (rather than initial) proportion of each species in the mixture is used as a basis for comparison; (3) RLO increases more steeply; and (4) the difference between mixture and monocultures widens as the stands develop.
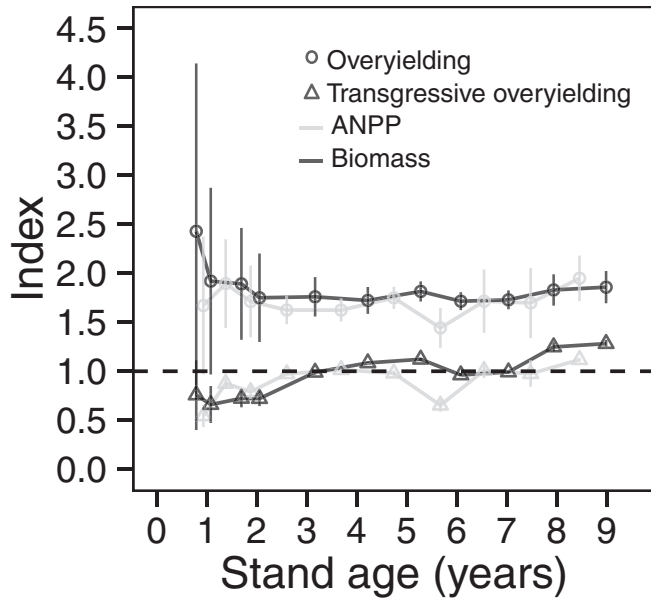
FIGURE 5. Overyielding and transgressive overyielding of mixtures calculated from biomass accrual (including roots) and aboveground net primary productivity (ANPP). Points are means; bars show range of possible values.

DISCUSSION

For the first time, we demonstrate steep age-dependent increases in the relative growth of a mixture of tree species compared with monocultures of its component species, whether measured by biomass accrual or ANPP. A cautionary reminder on generalization: Our results are based on a single suite of species selected, in part, for potential complementarity.

Our combination of morphologically and functionally different species overyielded every year (except the first). That the mixture did not convincingly or consistently overyield transgressively ($X_{mix}/X_{max} > 1$) is not unexpected: In the absence of an N-fixing species, transgressive overyielding is relatively infrequent and seldom significantly >1 (Trenbath 1974, Cardinale *et al.* 2007). Nevertheless, high, consistent overyielding indicates a biodiversity effect, which is some combination of resource partitioning, facilitation, or the chance inclusion of an exceptional performer, even in the absence of transgressive overyielding (Garnier *et al.* 1997, Špačková & Lepš 2001).

Was the biodiversity effect we observed the result of complementarity (*i.e.*, niche partitioning or facilitation), or was it the result of exceptionally fast growth by one or more species? We cannot discount completely the possibility that the enhanced performance of the mixture was due to faster growth in mixture by *Hieronyma* and *Cordia* due to relaxed intraspecific competition. Nevertheless, the combined evidence of survival of all five species in the mixture (Fig. 1A), the substantial excess of positive over negative deviations in biomass (Fig. 4), and consistently high overyielding by the mixture (Fig. 5) indicate that the biodiversity effect observed was likely due, in part at least, to complementarity.
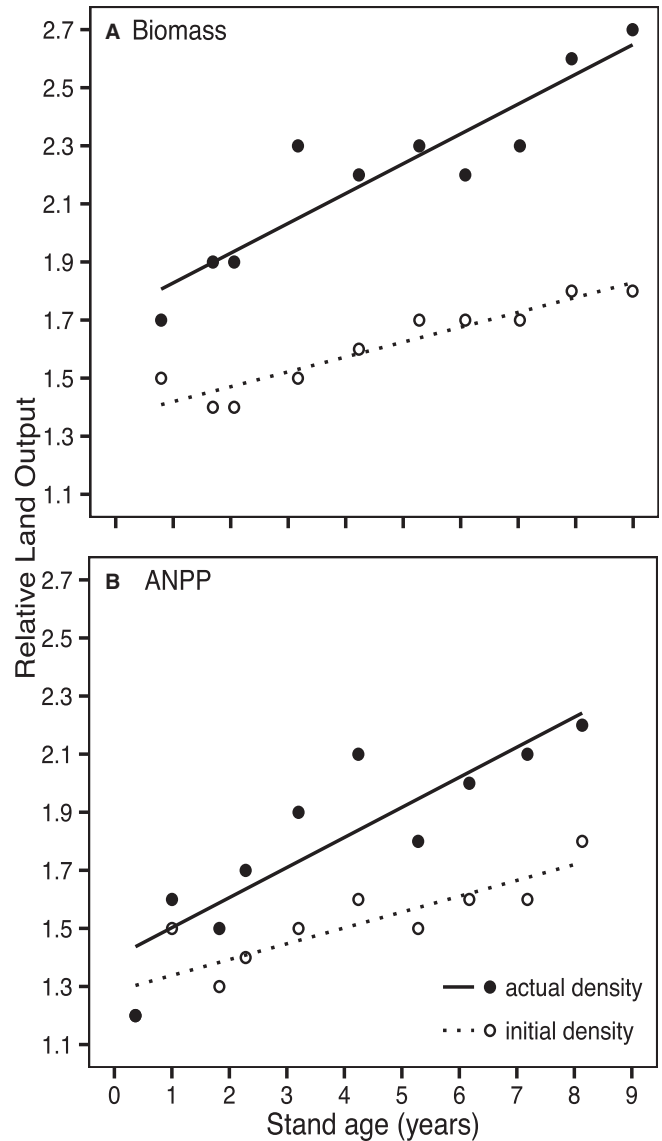

FIGURE 6. Comparative 9-yr performance of mixtures and monocultures of their component species. (A) Relative Land Output (RLO) as measured by biomass accrual (including roots). (B) RLO as measured by aboveground net primary productivity (ANPP).

DENSITY.—Our experiment was unique in that we were able to sustain monoculture plant densities at, or close to, optimal levels (vide Huxley & Maingu 1978, Vandermeer 1989), and in the mixtures, no species were lost and no species dominated to the exclusion of others. Did the light thinning of the eudicot monocultures bias the outcome in favor of the mixtures? We believe it did not, for if thinning had reduced monoculture performance we would have seen thinning-related dips in time courses of basal area or biomass (Fig. 2), but there were none. Thus, we feel confident that monoculture performance was not penalized by thinning and that the contrary is more likely: the growth of an unthinned stand of densely packed, large-stature, modular organisms would have declined had the stands been left for self-thinning to take its toll.

There is ample evidence for this in the literature of both forestry and ecology (Ryan *et al.* 2004). Tree monoculture performance is determined by the ability of the entire stand to sustain maximum potential use of available resources, whether this is accomplished by few widely spaced individuals or many closely packed individuals.

The calculation of each year's RLO based on initial planting density in the mixture reflects both growth and survival in the mixture (and growth of the monoculture, assuming its density sustains peak performance). In contrast, RLO calculation based on the number of survivors at each inventory date isolates the effect of plant growth. The difference between the two measures is due to mortality. This reasoning rests on two assumptions: maintenance of optimal density in the monocultures (discussed above), and no intraspecific competition in the mixture. At the beginning of the study, conspecifics were ≥ 8 m apart, and it unlikely that they competed. Eventually, however, crowns and roots might have been affected by conspecifics, particularly *Hieronyma*, which had high survival and a broad crown. Thus, our measures of mixture performance in the last few years of the study may be slightly lower than would have been the case at wider spacing.

Calculation of RLO based on number of survivors defines its upper bound. As the stands aged, the differences in RLO based on initial planting density or on survivors at time of measurement increased (Fig. 6), reflecting continued attrition in the mixture (Fig. 1). The proportion of potential RLO attributable to mortality increased from about 0.22 to 0.31 (biomass) or 0 to 0.27 (ANPP) during the 9-yr period. It is tempting to attribute the increase in survival-based RLO to more space per individual and relaxed intraspecific competition. If so, individuals in the mixture should have been larger than conspecifics in the monocultures, but except for *Cordia* that was not the case (Table 1). Instead, positive interactions among species, such as niche partitioning or facilitation, likely contributed to the observed difference between the two measures of RLO.

TEMPORAL COMPONENT.—There is typically a time delay of 2–5 yr before temperate-zone grassland mixtures exhibit complementarity (Hooper & Dukes 2004, Cardinale *et al.* 2007, Fargione *et al.* 2007, Reich *et al.* 2012). A similar time delay occurred with our eudicot-palm mixture, whose growth did not deviate measurably from the monocultures until the third year. Given the large stature of trees and the time required for roots and crowns to partition available space, a delay is not surprising. It does, however, serve as a warning against judging the comparative performance of monocultures and diverse systems based solely on the first year or two of growth after establishment.

Increasing complementarity has been documented in temperate-zone grasslands (*e.g.*, Tilman *et al.* 2001, Spehn *et al.* 2005), but this is the first such report for arborescent species. In a recent analysis of two long-term grassland studies, Reich *et al.* (2012) warned that the increase in complementarity over time portends reduction of functioning should species be lost, even those first perceived to be redundant. This is a particularly cogent

warning for tropical forests, where many tree species exist at low densities. Furthermore, the modular growth of long-lived structure enables arborescent species to sustain their position in a competitive hierarchy for many years, and to eventually grow faster if released from competition. Species that survive in small numbers or grow slowly may not contribute substantively to whole-system biomass or productivity, but the potential future contributions of minor components to ecosystem functioning should not be discounted.

AN ECOLOGICAL COST OF DIVERSITY.—Differential performance of species in monocultures and mixtures is typically addressed through complementarity and competition. Nevertheless, other biological interactions sometimes play significant roles in shifting the competitive balance, none more striking than our experience with *Cedrela* whose biomass in mixture at 9 yr was only 1.7 Mg/ha, or <10 percent of the expected value of 19.1 Mg/ha. Concealment from pests by host immersion in a diverse mixture is an attractive proposition, but tests involving various host–pest combinations in forests and tree plantations (reviewed by Schowalter 1996) have yielded all three possible outcomes: protection conferred, increased vulnerability, and no effect. The same range of possibilities holds for the mahogany shoot borer, *Hypsipyla*, which has been particularly well investigated because of the economic value of several vulnerable genera in the Meliaceae, including *Cedrela* (Newton *et al.* 1993). Shoot borer attack is seldom fatal, but it does retard height growth, and trees that are attacked repeatedly often succumb to competition from neighbors.

Virtually, all individuals of *Cedrela* in our plots were attacked by *Hypsipyla*, whether the community was a monoculture or a mixture of five species. What differed among treatments, however, was the post-attack response. In the five-species systems, each *Cedrela* was surrounded by different species, and these quickly overtopped the debilitated *Cedrela*, eventually resulting in the death of >95 percent of the original population (Fig. 1A). In the *Cedrela* monocultures, however, the growth of all individuals was retarded by approximately the same amount, so adjacent individuals did not outgrow their neighbors, and mortality was much lower. The *Cedrela* example, with its negative survival and growth responses to diversity, is a good reminder that the end result is not invariably determined by the susceptibility of the individual tree to herbivore damage; instead, it can be controlled by the vulnerability and response of neighbors.

POSSIBLE MECHANISMS.—Complementarity is expected to be strongest in harsh environments (Morin *et al.* 2011), yet we observed a substantial positive effect of diversity in one of the world's least-constraining terrestrial environments. In the absence of direct measures of total resource use, what can we deduce about possible resource partitioning or facilitation in the mixed-species plots? The three resources most likely to have been responsible were light, N, and phosphorus (P).

Given that our study-site soil was nutrient rich, well watered (by high rainfall), and well drained, it is unlikely that

nutrient or water availability figured as strongly in complementarity as has been reported for other studies (*e.g.,* Reich *et al.* 2012, Lebourgeois *et al.* 2013). Light was probably the greatest limitation to growth of all species, as indicated in other studies of trees. For example, Kittredge (1988), working in natural, mixed-species stands in the temperate zone, determined that trees in lower strata did not reduce growth of canopy species, and their growth represented additive productivity. He concluded that the primary factor regulating growth of overstory species was the degree of competition for aboveground growing space. In contrast, but not conflict, with that interpretation, Morin *et al.* (2011), using a gap-model approach involving mixtures of up to 30 species, concluded that competition for light induced a positive effect of species richness on productivity.

In our study, aboveground space was partitioned by crowns of different conformations, both horizontal and vertical. For example, the palms were deliberately included in the mixture because their foliage is vertical and arching, with many overlapping layers and high leaf area per plant. The eudicots, in contrast, ranged from tall, narrow crowns (*Cordia*) to deep, dense crowns (*Hieronyma*). We know, from adjacent three-species plots planted additively, that there were strong competitive effects caused by the palm growing upward into the bottom of the crowns of the two deciduous eudicots (*Cordia* and *Cedrela*), thereby reducing the eudicots' leaf area and light-capturing capacity (Ewel & Mazzarino 2008). The evergreen *Hieronyma*, in contrast, was not as vulnerable to competition for crown space from below, and it co-existed with *E. oleracea*. We suggest that, in our five-species mixture, the wider initial intraspecific spacing (compared with monocultures) led to relaxed intraspecific competition for *Hieronyma* and *Cordia*, which partitioned aboveground space by having very different crown forms. The other three species (*Cedrela* and the two *Euterpe* spp.) suffered when exposed to interspecific competition and although they survived, they contributed little to productivity. Their presence contributed significantly to diversity, but little to overyielding.

Light may not have been the only limiting resource in every instance, however, for there is evidence for spatial partitioning of soil by the two species that had highest survival (87% and 88% for *E. oleracea* and *Hieronyma* respectively at 13+ yr). In adjacent three-species plots, roots of *Hieronyma* completely dominated the uppermost 25 cm of soil, whereas those of *E. oleracea* were more abundant at greater depths (and extended to >3 m deep) (Ewel & Mazzarino 2008). This spatial partitioning may simply reflect interference competition; alternatively, it may have given the palm preferential access to deeply leached nutrients, particularly nitrate-N (Ewel & Bigelow 2011).

Alternatively, a mechanism related to facilitation might underlie some of the results. Previous work at the site demonstrated that plant-available soil P (0–10 cm) increased under *Hieronyma* (Ewel *et al.* 2014). This P enrichment could have come about via one or both of two proposed mechanisms, the first documented and the second hypothesized, in Ewel *et al.* (2014): deep-pumping of P and its return to the soil surface as litter, or P release from apatite facilitated by acidic organic exudates from *Hieronyma* litter or roots. The increased P under *Hieronyma* would have been potentially available to neighboring trees and could have facilitated their growth and survival.

ACKNOWLEDGMENTS

LITERATURE CITED

BALVANERA, P., A. B. PFISTERER, N. BUCHMANN, J. S. HE, T. NAKASHIZUKA, D. RAFFAELLI, AND B. SCHMID. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecol. Lett. 9: 1146–1156.

BROWN, A. 1992. Functioning of mixed-species stands at Gisburn, N.W. England. In M. G. R. Cannell, D. C. Malcolm, and P. A. Robertson (Eds.). The ecology of mixed-species stands of trees, pp. 125–150. Blackwell, Oxford, UK.

CALDWELL, M. M. 1987. Plant architecture and resource competition. In E. D. Schulze and H. Zwölfer (Eds.). Potentials and limitations of ecosystem analysis, pp. 164–179. Springer, Berlin Heidelberg.

CARDINALE, B. J., K. L. MATULICH, D. U. HOOPER, J. E. BYRNES, E. DUFFY, L. GAMFELDT, P. BALVANERA, M. I. O'CONNOR, AND A. GONZALEZ. 2011. The functional role of producer diversity in ecosystems. Am. J. Bot. 98: 572–592.

CARDINALE, B. J., D. S. SRIVASTAVA, J. E. DUFFY, J. P. WRIGHT, A. L. DOWNING, M. SANKARAN, C. JOUSEAU, M. W. CADOTTE, I. T. CARROLL, J. J. WEIS, A. HECTOR, AND M. LOREAU. 2009. Effects of biodiversity on the functioning of ecosystems: a summary of 164 experimental manipulations of species richness. Ecology 90: 854.

CARDINALE, B. J., J. P. WRIGHT, M. W. CADOTTE, I. T. CARROLL, A. HECTOR, D. S. SRIVASTAVA, M. LOREAU, AND J. J. WEISS. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. Proc. Natl. Acad. Sci. USA 104: 18123–18128.

CLARK, D. A., AND D. B. CLARK. 1992. Life history diversity of canopy and emergent trees in a neotropical rain forest. Ecol. Monogr. 62: 315–344.

COLE, T. G., AND J. J. EWEL. 2006. Allometric equations for four valuable tropical tree species. For. Ecol. Manage. 229: 351–360.

COUSENS, R., P. BRAIN, J. T. O'DONOVAN, AND P. A. O'SULLIVAN. 1987. The use of biologically realistic equations to describe the effects of weed density and relative time of emergence on crop yield. Weed Sci. 35: 720–725.

ERSKINE, P. D., D. LAMB, AND M. BRISTOW. 2006. Tree species diversity and ecosystem function: can tropical multi-species plantations generate greater productivity? For. Ecol. Manage. 233: 205–210.

EWEL, J. J., AND S. W. BIGELOW. 2011. Tree species identity and interactions with neighbors determine nutrient leaching in model tropical forests. Oecologia 164: 1127–1140.

EWEL, J. J., AND M. J. MAZZARINO. 2008. Competition from below for light and nutrients shifts productivity among tropical species. Proc. Natl. Acad. Sci. USA 105: 18836–18841.

EWEL, J. J., M. J. MAZZARINO, AND G. CELIS. 2014. Soil changes in model tropical ecosystems: effects of stand longevity outweigh plant diversity and tree species identity in a fertile volcanic soil. Ecosystems 17: 820–836.

FARGIONE, J., D. TILMAN, R. DYBZINSKI, J. H. R. LAMBERS, C. CLARK, W. S. HARPOLE, J. N. H. KNOPS, P. B. REICH, AND M. LOREAU. 2007. From selection to complementarity: shifts in the causes of biodiversity-productivity relationships in a long-term biodiversity experiment. Proc. Roy. Soc. B 274: 871–876.

FORRESTER, D. I. 2013. The spatial and temporal dynamics of species interactions in mixed-species forests: From pattern to process. For. Ecol. Manage. 312: 282–292.

GARBER, S. M., AND D. A. MAGUIRE. 2004. Stand productivity and development in two mixed-species spacing trials in the Central Oregon Cascades. For. Sci. 50: 91–105.

GARNIER, E., M. L. NAVAS, M. P. AUSTIN, J. M. LILLEY, AND R. M. GIFFORD. 1997. A problem for biodiversity-productivity studies: how to compare the productivity of multispecific plant mixtures to that of monocultures? Acta Oecol. 18: 657–670.

GRUBB, P. J., D. KELLY, AND J. MITCHLEY. 1982. The control of relative abundance in communities of herbaceous plants. In E. I. Newman (Ed.). The plant community as a working mechanism, pp. 79–97. Blackwell Scientific, Oxford, UK.

HARPER, J. L. 1977. Population biology of plants. Academic Press, London, UK.

HECTOR, A., B. SCHMID, C. BEIERKUHNLEIN, M. C. CALDEIRA, M. DIEMER, P. G. DIMITRAKOPOULOS, J. A. FINN, H. FREITAS, P. S. GILLER, J. GOOD, R. HARRIS, P. HÖGBERG, K. HUSS-DANELL, J. JOSHI, A. JUMPPONEN, C. KÖERNER, P. W. LEADLEY, M. LOREAU, A. MINNS, C. P. H. MULDER, G. O'DONOVAN, S. J. OTWAY, J. S. PEREIRA, A. PRINZ, D. J. READ, M. SCHERER-LORENZEN, E.-D. SCHULZE, A.-S. D. SIAMANTZIOURAS, E. M. SPEHN, A. C. TERRY, A. Y. TROUMBIS, F. I. WOODWARD, S. YACHI, AND J. H. LAWTON. 1999. Plant diversity and productivity experiments in European grasslands. Science 286: 1123–1127.

HOOPER, D. U., F. S. CHAPIN, III, J. J. EWEL, A. HECTOR, P. INCHAUSTI, S. LAVOREL, J. H. LAWTON, D. M. LODGE, M. LOREAU, S. NAEEM, B. SCHMID, H. SETÄLÄ, A. J. SYMSTAD, J. VANDERMEER, AND D. A. WARDLE. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecol. Monogr. 75: 3–35.

HOOPER, D. U., AND J. S. DUKES. 2004. Overyielding among plant functional groups in a long-term experiment. Ecol. Lett. 7: 95–105.

HUSTON, M. A., AND A. C. MCBRIDE. 2002. Evaluating the relative strengths of biotic versus abiotic controls on ecosystem processes. In M. Loreau, S. Naeem, and P. Inchausti (Eds.). Biodiversity and ecosystem functioning: synthesis and perspectives, pp. 36–46. Oxford University Press, Oxford, UK.

HUXLEY, P. A., AND A. MAINGU. 1978. Use of a systematic spacing design as an aid to the study of inter-cropping: some general considerations. Exp. Agr. 14: 49–56.

JOLLIFFE, P. A. 1988. Evaluating the effects of competitive interference on plant performance. J. Theor. Biol. 130: 447–459.

JOLLIFFE, P. A. 1997. Are mixed populations of plant species more productive than pure stands? Oikos 80: 595–602.

KITAJIMA, K., AND L. POORTER. 2008. Functional basis for resource niche partitioning by tropical trees. In W. P. Carson and S. A. Schnitzer (Eds.). Tropical forest community ecology, pp. 160–181. Wiley-Blackwell, Oxford, UK.

KITTREDGE, D. B. 1988. The influence of species composition on the growth of individual red oaks in mixed stands in southern New England. Can. J. For. Res. 18: 1550–1555.

LEBOURGEOIS, F., N. GOMEZ, P. PINTO, AND P. MÉRIAN. 2013. Mixed stands reduce Abies alba tree-ring sensitivity to summer drought in the Vosges mountains, western Europe. For. Ecol. Manage. 303: 61–71.

MALÉZIEUX, E., Y. CROZAT, C. DUPRAZ, M. LAURANS, D. MAKOWSKI, H. OZIER-LAFONTAINE, B. RAPIDEL, S. DE TOURDONET, AND M. VALENTIN-MORISON. 2009. Mixing plant species in cropping systems: concepts, tools and models: a review. In E. Lichtfouse, M. Navarrete, P. Debake, V. Souchè, and C. Alberola (Eds.). Sustainable agriculture, pp. 329–353. Springer, New York City, New York.

MORIN, X., L. FAHSE, M. SCHERER-LORENZEN, AND H. BUGMANN. 2011. Tree species richness promotes productivity in temperate forests through strong complementarity between species. Ecol. Lett. 14: 1211–1219.

NELDER, J. A. 1962. New kinds of systematic designs for spacing experiments. Biometrics 18: 283–307.

NEWTON, A. C., P. BAKER, S. RAMNARINE, J. F. MESÉN, AND R. LEAKEY. 1993. The mahogany shoot borer: prospects for control. For. Ecol. Manage. 57: 301–328.

PIOTTO, D., D. CRAVEN, F. MONTAGNINI, AND F. ALICE. 2010. Silvicultural and economic aspects of pure and mixed native tree species plantations on degraded pasturelands in humid Costa Rica. New Forest. 39: 369–385.

POTVIN, C., L. MANCILLA, N. BUCHMANN, J. MONTEZA, T. MOORE, M. MURPHY, Y. OELMANN, M. SCHERER-LORENZEN, B. L. TURNER, W. WILCKE, F. ZEUGIN, AND S. WOLF. 2011. An ecosystem approach to biodiversity effects: carbon pools in a tropical tree plantation. For. Ecol. Manage. 261: 1614–1624.

QUIJAS, S., B. SCHMID, AND P. BALVANERA. 2010. Plant diversity enhances provision of ecosystem services: a new synthesis. Basic Appl. Ecol. 11: 582–593.

REICH, P. B., D. TILMAN, F. ISBELL, K. MUELLER, S. E. HOBBIE, D. F. B. FLYNN, AND N. EISENHAUER. 2012. Impacts of biodiversity loss escalate through time as redundancy fades. Science 336: 589–592.

ROTHE, A., AND D. BINKLEY. 2001. Nutritional interactions in mixed species forests: a synthesis. Can. J. For. Res. 31: 1855–1870.

RYAN, M. G., D. BINKLEY, J. H. FOWNES, C. P. GIARDINA, AND R. S. SENOCK. 2004. An experimental test of the causes of forest growth decline with stand age. Ecol. Monogr. 74: 393–414.

SCHOWALTER, T. 1996. Stand and landscape diversity as a mechanism of forest resistance to insects. In W. Mattson, P. Niemelä, and M. Rousi (Eds.). Dynamics of forest herbivory: quest for pattern and principle, pp. 21–27. USDA Forest Service, Saint Paul, Minnesota.

ŠPAÈKOVÁ, I., AND J. LEPŠ. 2001. Procedure for separating the selection effect from other effects in diversity–productivity relationship. Ecol. Lett. 4: 585–594.

SPEHN, E. M., A. HECTOR, J. JOSHI, M. SCHERER-LORENZEN, B. SCHMID, E. BAZELEY-WHITE, C. BEIERKUHNLEIN, M. C. CALDEIRA, M. DIEMER, P. G. DIMITRAKOPOULOS, J. A. FINN, H. FREITAS, P. S. GILLER, J. GOOD, R. HARRIS, P. HÖGBERG, K. HUSS-DANELL, A. JUMPPONEN, J. KORICHEVA, P. W. LEADLEY, M. LOREAU, A. MINNS, C. P. H. MULDER, G. O'DONOVAN, S. J. OTWAY, C. PALMBORG, J. S. PEREIRA, A. B. PFISTERER, A. PRINZ, D. J. READ, E.-D. SCHULZE, A.-S. D. SIAMANTZIOURAS, A. C. TERRY, A. Y. TROUMBIS, F. I. WOODWARD, S. YACHI, AND J. H. LAWTON. 2005. Ecosystem effects of biodiversity manipulations in European grasslands. Ecol. Monogr. 75: 37–63.

SPEHN, E. M., J. JOSHI, B. SCHMID, M. DIEMER, AND C. KÖRNER. 2000. Above-ground resource use increases with plant species richness in experimental grassland ecosystems. Funct. Ecol. 14: 326–337.

THOMPSON, K., A. P. ASKEW, J. P. GRIME, N. P. DUNNETT, AND A. J. WILLIS. 2005. Biodiversity, ecosystem function and plant traits in mature and immature plant communities. Funct. Ecol. 19: 355–358.

TILMAN, D., P. B. REICH, J. KNOPS, D. WEDIN, T. MIELKE, AND C. LEHMAN. 2001. Diversity and productivity in a long-term grassland experiment. Science 294: 843–845.

TRENBATH, B. R. 1974. Biomass productivity of mixtures. Adv. Agron. 26: 177–210.

VANDERMEER, J. H. 1989. The ecology of intercropping. Cambridge University Press, Cambridge, UK.