Forest Ecology and Management 261 (2011) 1614–1624

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

An ecosystem approach to biodiversity effects: Carbon pools in a tropical tree plantation

Catherine Potvin[a,b,*], Lady Mancilla[b], Nina Buchmann[c], Jose Monteza[b], Tim Moore[g], Meaghan Murphy[g], Yvonne Oelmann[d], Michael Scherer-Lorenzen[f], Benjamin L. Turner[b], Wolfgang Wilcke[e], Fabienne Zeugin[c], Sebastian Wolf[c]

[a] Department of Biology, McGill University, 1205 Dr Penfield, Montreal, Canada H3A 1B1
[b] Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancón, Panama
[c] Institute of Plant, Animal and Agroecosystem Sciences, ETH Zurich, Universitaetsstrasse 2, 8092 Zurich, Switzerland
[d] Institute of Integrated Natural Sciences, Geography, University of Koblenz-Landau, Universitätsstr. 1, 56070 Koblenz, Germany
[e] Geographic Institute, University of Berne, Hallerstrasse 12, 3012 Berne, Switzerland
[f] Faculty of Biology, Albert-Ludwigs University of Freiburg, Schaenzlestrasse 1, 79104 Freiburg, Germany
[g] Department of Geography, McGill University, 805 Sherbrooke Street West, Montreal, Quebec, Canada H3A 2K6

ARTICLE INFO

Article history:
Received 15 July 2010
Received in revised form 4 November 2010
Accepted 15 November 2010
Available online 16 December 2010

Keywords:
Tropical tree plantation
Carbon pools and fluxes
Diversity effect
Ecosystem carbon storage

ABSTRACT

This paper presents a synthesis of experiments conducted in a tropical tree plantation established in 2001 and consisting of 22 plots of 45 m × 45 m with either one, three or six native tree species. We examined the changes in carbon (C) pools (trees, herbaceous vegetation, litter, coarse woody debris (CWD), and mineral topsoil at 0–10 cm depth) and fluxes (decomposition of CWD and litter, as well as soil respiration) both through time and among diversity levels. Between 2001 and 2009 the aboveground C pools increased, driven by trees. Across diversity levels, the mean observed aboveground C pool was 7.9 ± 2.5 Mg ha^{-1} in 2006 and 20.4 ± 7.4 Mg ha^{-1} in 2009, a 158% increase. There was no significant diversity effect on the observed aboveground C pool, but we found a significant decrease in the topsoil C pool, with a mean value of 34.5 ± 2.4 Mg ha^{-1} in 2001 and of 25.7 ± 5.7 Mg ha^{-1} in 2009 ($F_{1,36} = 52.12$, $p < 0.001$). Assuming that the biomass C pool in 2001 was negligible (<1 Mg ha^{-1}), then the plantation gained in C, on average, ~20 and lost ~9 Mg ha^{-1} in biomass and soil respectively, for an overall gain of ~11 Mg ha^{-1} over 8 years. Across the entire data set, we uncovered significant effects of diversity on CWD decomposition (diversity: $F_{2,393} = 15.93$, $p < 0.001$) and soil respiration (monocultures vs mixtures: $t = 15.35$, df = 11, $p < 0.05$) and a marginally significant time × diversity interaction on the loss of total C from the mineral topsoil pool (see above). Monthly CWD decomposition was significantly faster in monocultures (35.0 ± 24.1%) compared with triplets (31.3 ± 21.0%) and six-species mixtures (31.9 ± 26.8%), while soil respiration was higher in monocultures than in mixtures ($t = 15.35$, df = 11, $p < 0.001$). Path analyses showed that, as diversity increases, the links among the C pools and fluxes strengthen significantly. Our results demonstrate that tree diversity influences the processes governing the changes in C pools and fluxes following establishment of a tree plantation on a former pasture. We conclude that the choice of tree mixtures for afforestation in the tropics can have a marked influence on C pools and dynamics.

1. Introduction

An important challenge of climate change mitigation is the management of terrestrial carbon (C) either to create new C sinks or to preserve existing ones (Malhi et al., 1999). In this context, a number of studies have recently compared the productivity of mixed-species plantations with monocultures to test whether diversity would enhance productivity and hence C storage (Caspersen and Pacala, 2001; Vila et al., 2007; Firn et al., 2007; Schlapfer and Schmid, 1999; Piotto et al., 2010; Erskine et al., 2006). Tree plantations and agroforests are widely believed to enhance the terrestrial C pool (Nair et al., 2009b) and reforestation with native species could potentially yield a range of additional benefits, including soil stabilization, reduced erosion, habitat for a variety of species including birds, seed deposition, and increased understory diversity (Wishnie et al., 2007). Nevertheless, there is a growing concern that tree plantations, whether with natives or exotics, might decrease water availability at the ecosystem level (Malmer et al., 2010).

* Corresponding author at: Department of Biology, McGill University, 1205 Dr Penfield, Montreal, Quebec, Canada H3A 1B1.
E-mail address: catherine.potvin@mcgill.ca (C. Potvin).

0378-1127/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.foreco.2010.11.015

Many studies examining the effect of tree diversity on productivity or C storage used aboveground tree biomass as a surrogate for net primary productivity (NPP: the total production of plant biomass within a given time period) (Catovsky et al., 2002). NPP is a good indicator of ecosystem C storage only if large-scale disturbances during a specific period can be ruled out, because C is lost through heterotrophic respiration, fire and other disturbances such as human harvest (Körner, 2000, 2003; Schlesinger and Lichter, 2001; Schulze et al., 2000). Ecosystem C storage is tightly coupled with changes in the soil that occur in response to alterations in above- and below-ground productivity, rooting depth and root distribution, and changes in the quality and quantity of litter (Catovsky et al., 2002; Nair et al., 2009b; Valverde-Barrantes, 2007). Assessment of ecosystem C storage must therefore include belowground C pools.

We compared several pools of C (standing tree biomass, coarse woody debris (CWD), herbaceous vegetation, litter and soil) and fluxes of C (soil respiration and the decomposition of CWD and litter) in a tropical tree plantation established with one, three or six native species. We hypothesized that tree diversity affects the soil C pool through its effect on inputs, mainly as CWD and litter, and that, therefore, tree diversity affects ecosystem C storage beyond its effect on aboveground NPP.

2. Methods

Measurements took place in a diversity plantation established near the village of Sardinilla in the region of Buena Vista, Panama (9°19′30″N, 79°38′00″W) between 2001 and 2009. In 2001, six native tree species were selected for planting, including two pioneers (*Luehea seemannii* Triana & Planch (Ls) and *Cordia alliodora* (Ruiz & Pavon) Oken (Ca)), two light-intermediate species (*Anacardium excelsum* (Bert. & Balb. Ex Kunth) Skeels (Ae) and *Hura crepitans* L. (Hc)) and two long lived pioneers (*Cedrela odorata* L. (Co) and *Tabebuia rosea* (Bertol.) DC. (Tr)) (Delagrange et al., 2008). The plantation consisted of 24 plots of approximately the same size (45 × 45 m). Twelve plots (two for each species) are monocultures, six plots contain different combinations of three tree species, and six plots contain all tree species (Scherer-Lorenzen et al., 2005b). The 24 diversity plots were embedded to the north, south and east, in a larger reforestation area using native tree species (∼10 ha total reforestation area), which reduced the edge effect in all but one cardinal direction. Undergrowth was cleared annually to eliminate other competing vegetation and facilitate work within the plantation. Each plot was distributed randomly to reduce bias caused by differences in soil conditions. Plots were square-shaped and established with an average of 231 trees planted at 3 m spacing. *Cordia alliodora* suffered significant mortality after planting, so monocultures of this species were excluded from the analyses.

2.1. Carbon pools

2.1.1. Aboveground tree biomass

Every year at the onset of the dry season (December–January), height, basal diameter at 10 cm from the ground, and diameter at breast height (DBH) of every stem were measured on each individual tree. These traits were used to compute the above-ground biomass of individual trees based on species-specific allometric regressions developed at the site in 2007 (Table 1). Because biomass production varied with tree age, we restricted our analysis to the years 2006, 2007 and 2009 to ensure the relevance of the allometric equations. The year 2008 was excluded because data on CWD were not available. The area of each plot varied slightly, ranging from 0.2025 to 0.2304 ha. It was therefore necessary to scale up biomass to 1 ha to compare stand-level tree biomass of the different diversity levels (Eq. (1)):

$$T_1 = A \cdot \left(\frac{(\sum b_i)}{N} \right) \quad (1)$$

where T_1 is aboveground tree biomass at the plot level, b_i is the biomass of each tree in the plot, N is the number of living trees in the plot, and A is the area of each plot.

Since planting, a number of trees have died in the plots and consequently plots have different numbers of trees ranging between 101 trees in plot A2 (one of the six-species plots) and 229 trees in plot Tr1 (monoculture of *Tabebuia rosea*). Potvin and Gotelli (2008) showed that tree mortality in Sardinilla was dependent on species and independent of diversity. To correct for mortality, a second estimate of tree biomass at the plot level was calculated using the following equation:

$$T_2 = 1111 \cdot \left(\frac{(\sum b_i)}{N} \right) \quad (2)$$

where T_2 is aboveground tree biomass at the plot level corrected for mortality and 1111 is the number of trees planted in a 1 ha reforestation plot with no mortality. Throughout the paper, we maintain the distinction between the estimates of biomass with (observed aboveground C pools) or without mortality (maximum aboveground C pool).

Tree biomass at the plot level was converted to tree C using species-specific trunk C concentration obtained from coring tree trunks in the vicinity (Hc: 45.07 g C kg^{-1}; Ls: 45.76 g C kg^{-1}; Ae: 45.82 g C kg^{-1}; Tr: 47.01 g C kg^{-1}; Co: 47.39 g C kg^{-1}) (Elias and Potvin, 2003). For species mixtures, the mean C concentration of the constituent species was used.

2.1.2. Coarse woody debris

All CWD within each plot was collected and weighed at the height of the dry season in March of 2006, 2007 and 2009. Each plot was searched by a team of people walking in a straight line between the trees and collecting all visible twigs, branches, and trunks belonging to the trees in that plot. Leaf litter and litter from the herbaceous understory were excluded. All materials were weighed on site and values were scaled to a hectare basis in the same way as tree biomass with or without correcting for tree mortality. Coarse woody debris biomass was then converted to C using the species-specific trunk C concentration.

2.1.3. Litter production

Yearly litter production at the plot level was estimated by combining two existing data sets. First, for the dry season, a total of 204 litter traps of 1 m^2 were positioned randomly in the plantation

Table 1
Best species specific least square linear regression between tree biomass and basal diameter (BD), height (H), the sum of the DBH of all stems (DBH_{all}) and wood.

Species	Allometric equation	Height range	R^2
Anacardium excelsum	$\log(\text{Biom}) = 0.73943 - 7.32969\,BD - 4.02612\,BD^2 - 0.49008BD^3$	0.9–8.4 m	0.9693
Cedrela odorata	$\log(\text{Biomass}) = -1.3288 - 10.278 \log BD - 5.517l\,(\log DB)^2 - 0.7624(\log BD)^3$	0.13–10.8 m	0.7685
Hura crepitans	$\log(\text{Biomass}) = 2.0279 + 1.8733 \log H + 1.0001 \log DBH_{all}$	0.9–10.3 m	0.884
Luhea seemannii	$\log(\text{Biomass}) = 0.2335 + 2.1608 \log H + 0.6082 \log DBH_{all}$	1–8.2 m	0.8826
Tabebuia rosea	$\log(\text{Biomass}) = 6.21017 + 1.1268 \log(BD^2HS)$	1.3–7.8 m	0.8646

with 12 traps per plot and three traps per subplot. To establish the subplots, each plot was separated into four equal sections. Randomizing per subplot ensured that litter traps were positioned throughout each plot. Litter collection took place between February and April 2005. Litter was collected on a bi-weekly basis and samples were dried before weighing as described in Scherer-Lorenzen et al. (2007). Litter production at the plot level was estimated for the four month dry season as follows:

$$\text{Litter}_{\text{dry}} = \left(P_j \cdot \frac{A_j}{N_j}\right) \cdot N_j \quad (3)$$

where P_j is litter production per area in plot j (g m^{-2}), A_j the area of plot j (m), N_j is the number of trees in plot j, N_j was either the number of living trees in plot j, or 1111, which is the number of trees in a standard 1-ha plantation, depending on whether the estimate of litter production at the plot level controlled for tree mortality or not.

Litter was also collected on a bi-weekly basis between July and November in 2007 and 2008. In these sampling campaigns, the same 1 m^2 litter traps were positioned under each of 60 individual trees growing in each of the diversity levels (Oelmann et al., 2010) rather than in subplots as in the dry season experiment. The data were used to estimate litter biomass in the wet season. Contrary to our expectation that litter production should increase with the age of the plantation, litter production was not significantly higher in the wet season of 2008 than in 2007. We therefore used the average litter production of these two years in our estimate of yearly litter production. The scaling up of wet season litter production was based on species identity and the number of trees per species in each plot:

$$\text{Litter}_{\text{wet}} = \left(\frac{\sum_k (X_{ij} N_{ij})}{A_j}\right) \cdot N_j \quad (4)$$

where X_{ij}s the mean litter production for 2007 and 2008 of species i in plot j, N_{ij} is the number of trees of species i in plot j, N_j was either the number of living trees in plot j or 1111, which is the number of trees in a standard 1 ha plantation depending on whether the estimate of litter production at the plot level controlled for tree mortality or not.

An approximate estimate of yearly litter biomass was calculated as the sum of dry and wet season litter production, assuming that the dry season lasted 4 months and the wet season lasted 8 months. Months for which we had no data (January, May and June), were filled by the average monthly litter biomass for the dry and wet season mean production, respectively:

$$\text{Total litter} = \left(4 \cdot \left(\frac{\text{Litter}_{\text{dry}}}{3}\right)\right) + \left(8 \cdot \left(\frac{\text{Litter}_{\text{wet}}}{5.5}\right)\right) \quad (5)$$

This assumption seems plausible, because some species (e.g. *Anacardium excelsum*) shed leaves all year round. The Sardinilla plantation includes ten monoculture plots (i.e., two replicates per species). Unfortunately, litter was collected in different monocultures of the same species in the two seasons. Therefore, litter biomass in monoculture is a composite estimate of litter production from the two monocultures of the same species rather than a plot specific value:

$$\text{Total litter}_{\text{m}} = \left(4 \cdot \left(\frac{\text{Litter}_{\text{dry}\,i}}{3}\right)\right) + \left(8 \cdot \left(\frac{\text{Litter}_{\text{wet}\,j}}{5.5}\right)\right) \quad (6)$$

where $\text{Litter}_{\text{dry}\,i}$ refers to the litter production of the first monoculture of a given species, and $\text{Litter}_{\text{wet}\,j}$ refers to the litter production of the second monoculture of a given species. Finally, litter biomass was converted into litter C pool by using plot- and species-specific C concentrations measured from the dry season litter (Scherer-Lorenzen et al., 2007).

Mean litter production per tree of 233 ± 60 g and 122 ± 15 g, in the wet season of 2007 and 2008 respectively, suggests that although trees allegedly grow through time, factors other than years determine litter C pool. We therefore calculated a single estimate of the litter C pool and used it in the calculation of the aboveground C pool in 2006, 2007 and 2009 assuming no increase in litter production between 2006 and 2009.

2.1.4. Herbaceous biomass

To estimate the biomass of the herbaceous vegetation, each plot was divided into four subplots, giving a total of 96 subplots. Within each subplot, herbaceous vegetation was cut to ground level in one randomly positioned, non-permanent, quadrat (0.5 m^2) twice a year at the beginning and end of the wet season (May/June and November/December). The green and dry (litter) biomass was separated, dried and weighed. The dry and green biomass within each of the four quadrats was summed and scaled up to the plot level, taking into account individual plot size. Herbaceous biomass was then scaled to 1 ha. Here we report data of 2006, 2007 and 2009.

In addition, to estimate the C concentration, herbaceous vegetation was collected with a frame (0.06 m^2) within the area confined by the canopy drip line under each of the 60 focal trees used to estimate litter production in the wet season. The herbaceous vegetation was sorted into grasses and non-leguminous herbs (henceforward termed grasses/herbs) and legumes and the respective weight was recorded. For all samples, the C concentration was measured with an elemental analyzer (Vario EL III, Elementar Analysensysteme, Hanau, Germany). We found no significant diversity effect on C concentration of legumes or grasses/herbs, but the proportion of grasses/herbs was significantly higher ($F_{2,24} = 5.23$, $p < 0.01$) in triplets (69.3%) than in monoculture (46.4%) and six-species mixtures (44.8%). To transform herbaceous biomass into herbaceous C pool, we therefore used the following index:

$$C_{\text{herbs}} = (40.66 \cdot \text{Prop}_{\text{g/h},i}) + (44.78 \cdot \text{Prop}_{\text{l},i}) \quad (7)$$

where 40.66% and 44.78% are the mean C concentration of, respectively, grasses/herbs and legumes under the 60 focal trees, $\text{Prop}_{\text{g},i}$ is the mean proportion of grasses/herbs in each diversity level, and $\text{Prop}_{\text{l},i}$ is the mean proportion of legumes in each of the diversity levels.

2.1.5. Soil measurements

Initial soil sampling was done in July 2001 during plantation establishment, both at the site of the plantation and in an adjacent pasture. This included 225 topsoil samples (0–10 cm) and seven soil profiles (0–100 cm), using a 10 cm cylindrical corer with a diameter of 5 cm (Abraham, 2004). Soil sampling of topsoil (0–10 cm) was repeated in March 2009 using a 10 cm cylindrical corer with a diameter of 6.8 cm and sampling once per plot ($n = 22$). Samples were dried for at least 72 h in a drying room at 60 °C and were afterwards analyzed for soil organic C concentration (SOC) and δ^{13}C with a Flash 1112 Elemental Analyzer coupled to a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, USA). Bulk density (d_B) sampling in March 2009 (dry season) resulted in higher values than the sampling in July 2001 (wet season) by Abraham (2004). However, we assume that this is related to seasonal soil contraction due to the large clay content than to inherent changes in d_B. A study by Seitlinger (2008) with sampling in June 2007 (wet season) found similar d_B values to Abraham (2004). Assuming no change in d_B from 2007 to 2009, we used the d_B values reported by Seitlinger (2008) to calculate the soil organic C pool (SOCP) from the C concentrations from samples taken in 2009. To derive the contribution of the source of organic matter in the topsoil, we used a two-member mixing model and the reported values by Abraham (2004), with −14.4‰ for pasture

litter from the Sardinilla site and −29.5‰ for tree litter obtained from nearby Barro Colorado Island.

2.2. Fluxes

2.2.1. Decomposition of coarse woody debris

Decomposition of CWD was measured between May 2007 and March 2008 for medium size CWD (~2 cm diameter) for each species in each diversity treatment. Dead branches were collected on the ground in April 2007 and cut into 10 cm stakes. Twenty stakes were cut, weighed, dried and re-weighed to serve as control for no decomposition. Mesh bags were made of mosquito screen (1 mm mesh size) and 10 stakes from one species were put into bags. The stakes that were put in the mesh bags were not dried to ensure that the chemical composition was not changed. Each of the stakes was weighed and identified with a metal tag. For each species, 12 mesh bags were prepared and positioned on the ground at the centre of two randomly chosen subplots of the target species' two monocultures, of two of the triplets where this target species grew and of two randomly chosen six-species plots.

The decomposition of a total of 120 stakes per species was therefore followed through time. Every month, one stake was removed from each mesh bag and both the fresh and dry masses were recorded to calculate the loss in woody biomass. For each species in each diversity level, we calculated the decomposition rate constant, k, from the decay model (Scherer-Lorenzen et al., 2007) over the initial six months period, June to December 2007:

$$X = X_0 \cdot e^{-kt} \tag{8}$$

where X is the mass of CWD remaining at time t, X_0 is the initial mass, k is the exponential decay coefficient, and t is time in months. While it is possible that the mesh bags could have decreased the rate of decomposition by excluding some detritivorous arthropods, the method is recommended by the Long-term Intersite Decomposition Experiment Team (LIDET) (Harmon and Sexton, 1996).

2.2.2. Litter decomposition

Litter decomposition was studied using litter bags as described in Scherer-Lorenzen et al. (2007). Briefly, litter bags (1 mm mesh size) were filled with 10 g of dry litter with the proportion of each species representing their abundance within each plot. Replicate litter bags were retrieved every four weeks during a 116-day period from May to September 2005, corresponding to the wet season, and loss in dry weight was determined.

2.2.3. Soil respiration

Soil respiration measurements were made using a closed chamber system and a PP Systems (Amesbury, MA, USA) EGM-4 Environmental infrared gas analyzer with collars permanently inserted into the ground to 5 cm depth one month prior to sampling. We built a PVC soil respiration chamber of 20.32 cm diameter and 10.16 cm height for a total volume of 3293 cm^3. To capture seasonal changes in soil respiration, each collar was sampled up to twice per week over four weeks in March 2004 at the height of the dry season, and again in June 2004 at the onset of the wet season. Soil respiration was measured in the area between two trees in both monoculture and mixture pairs. Overall, we measured respiration of monoculture pairs for all six species and of 12 unique two-species pairs. Monoculture pairs were selected among their corresponding monoculture plots, while two-species pairs were selected randomly from either the triplets or the six-species mixtures (see Murphy et al., 2008 for methodological details). With six monoculture pairs and 12 two-species pairs, each replicated five times, we sampled a total of 90 collars in the biodiversity plots.

Using the combined March and June soil respiration data, we modeled plot level respiration of the six-species mixtures. As a null hypothesis we considered plot respiration as an additive function of the number of individuals of each species, where the respiration of each species in the plot was calculated from the respiration values of each species in monoculture. Summing the respiration of each species produced a value for plot level respiration (model 1; Eq. (9)):

$$R_{1j} = X_j \cdot N_j \tag{9}$$

where R_{1j} is the respiration of species j in the plot, X is the average respiration of species j in monoculture (1–6), N_j is the number of individuals of species j, and:

$$B_{1m} = \sum R_{i(1-6)} \tag{10}$$

where B_{1m} is the respiration of six-species plot estimated independently for each of the six plots.

Model 2 assumes that plot level respiration is dependent upon the interactions between individual trees and their neighbors of different species. Individual tree respiration is calculated as follows:

$$R_{2i} = X_{ia} \cdot N_{ia} + X_{ib} \cdot N_{ib} \tag{11}$$

where R_{2i} is the respiration of individual i (1–225), x_{ia} is the average respiration of individual i paired with neighbor a, x_{ib} is the average respiration of individual i paired with neighbor b, N_{ia} is the number of individual i with neighbors (a) (1 and 2), and N_{ib} is the number of individual i with neighbors (b) (1 and 2). The sum of all individual tree respiration rates is the plot level respiration rate:

$$B_2 = \sum R_{2(1-225)} \tag{12}$$

To account for spatially explicit differences in the plots, calculations were repeated for each of the six-species plots.

2.3. Calculation and statistical analyses

We estimated NPP as the change in aboveground biomass of four different biomass compartments (trees, litter, herbaceous and CWD) between consecutive years. NPP was therefore calculated from biomass increment for the 2006–2007 (NPP_{2007}) and the 2007–2009 (NPP_{2009}) intervals.

Aboveground C pool size was calculated for each plot in 2006, 2007 and 2009 by summing the C mass of the following ecosystem components: CWD, trees, herbaceous vegetation and litter. These components were scaled up either as observed (i.e., scaled up with existing tree density; observed aboveground C pools), or as maximum (i.e., scaled up assuming 1111 trees per ha; Maximum aboveground C pools). We therefore obtained two measures of ACP for each plot. The relative growth rates of both estimates of ACP were calculated for each plot between 2006 and 2009:

$$\mathrm{RGR_{ACP}} = \frac{(\ln(\mathrm{ACP_2}) - \ln(\mathrm{ACP_1}))}{t} \tag{13}$$

where time interval (t) is defined as a function of the date of two successive measurements: $\mathrm{date_2} - \mathrm{date_1}/365.25$ or $\mathrm{date_2} - \mathrm{date_1}/730.5$, respectively, for one or two years interval.

We also calculated ecosystem C storage as the sum of aboveground C pool, root C and the topsoil SOC pool (SOCP). Root biomass and associated C were estimated using a site and species-specific root/shoot ratio obtained by excavating entire root systems in 2003 in trees planted previously in 1998 in an adjacent plantation (Coll et al., 2008) and harvested at a similar age at the time of excavation. The root/shoot ratios were 0.29 (Hc), 0.30 (*Ls*), 0.37 (Ae, Tr) and 0.55 (Co). Finally, ecosystem C gain between 2001 and 2009 was estimated as:

$$\mathrm{Ecosystem\ \ C\ \ gain} = \mathrm{ACP_{2009}} + \mathrm{RC_{2009}} + (\mathrm{SOCP_{2009}} - \mathrm{SOCP_{2001}}) \tag{14}$$

where ACP_{2009} is aboveground C pool, i.e., the sum of the C mass of CWD, trees, herbaceous vegetation and litter in 2009, RC_{2009} is the root C pool estimated from root/shoot ratio for 2009, and $SOCP_{2009} - SOCP_{2001}$ is the difference in the topsoil organic C pools between 2001 and 2009 and takes a negative value.

Litter C concentration, ecosystem C gain and RGR of C were analyzed by univariate ANOVA (Type III Sums of Squares) with diversity as the main effect of interest. Aboveground ecosystem characteristics measured repeatedly at the plot level through time (e.g. C pools in trees, CWD, herbaceous vegetation as well as aboveground C pool), were tested using an ANOVA with repeated measures (ANOVAR) with diversity as the main between subject effect and years as the main within subject effect. Soil characteristics measured in 2001, prior to the establishment of the plantation, and in 2009 were analyzed by two-way ANOVAs with year, diversity and their interactions as the effects of interest secause sampling did not occur at exactly the same position in 2001 and 2009, which prevented us from using ANOVAR. Decomposition of CWD was analyzed by three-way ANOVA with diversity, species and month as the main factors. Because measurements were replicated at the subplot level, the model considered that the appropriate error for diversity was plot (diversity). Month was not considered as a repeated factor since a different bag was measured each time. Pearson's correlation coefficient was used to test the relationship between different C pools and fluxes. For soil respiration data, the assumption of no diversity effect (i.e., $B_1 = B_2$; Eqs. (8) and (10)), was tested using a paired *t*-test with plot-level soil respiration rates for each of the six-species plots estimated by both methods (B_1 and B_2).

Finally, for 2009, we tested for three different path models to relate the observed aboveground C pools and SOC. These paths were:

$$\mathrm{SOC} = \mathrm{Trees} + \mathrm{Herbaceous} + \mathrm{CWD} + \mathrm{Litter} + \varepsilon \quad (15)$$

$$\mathrm{SOC} = \mathrm{RGR}_{\mathrm{Trees}} + \mathrm{Herbaceous} + \mathrm{CWD} + \mathrm{Litter} + \varepsilon \quad (16)$$

$$\Delta\mathrm{SOC} = \mathrm{RGR}_{\mathrm{Trees}} + \mathrm{Herbaceous} + \mathrm{CWD} + \mathrm{Litter} + \varepsilon \quad (17)$$

Data were standardized prior to the analysis. All statistical analyses were performed using SAS v. 9.2. Normality was checked prior to the analysis. The only variable that needed transformation was litter production, which was normalized by calculating the square-root.

3. Results

3.1. Carbon pools

3.1.1. Net primary production and aboveground pools

Overall, NPP ranged from 14.9 Mg ha^{-1} yr^{-1} in monocultures in 2007 to 23.6 Mg ha^{-1} yr^{-1} in triplets in 2009. Year was the only significant difference observed with an increase through time overall, NPP_{2009} being significantly higher (21.4 ± 2.1 Mg ha^{-1} yr^{-1}) than NPP_{2007} (15.5 ± 1.0 Mg ha^{-1} yr^{-1}). Observed aboveground C pools were estimated yearly as the sum of trees, CWD, litter and herbaceous C pools for each diversity level. Not surprisingly, the ANOVA unveiled a significant effect of year ($F_{2,38} = 93.48$, $p < 0.0001$), with C pools increasing from 2006 to 2009 (Fig. 1). Across diversity levels, the general mean for observed aboveground C pools in 2006 were 7.9 ± 2.5 Mg ha^{-1}, while in 2009 the observed aboveground C pool was 20.4 ± 7.4 Mg ha^{-1}, a 158% increase. The effect of diversity on the observed aboveground C pools was not significant.

In 2009, the contribution of the four components of aboveground C pools ranked CWD ~ litter < herbaceous < trees (Table 2). Living trees accounted for 59–84% of the total observed aboveground C pool. The herbaceous C pool was an important component of the system, with between 3.6 and 6.3 Mg ha^{-1} among the different plots in 2009. The contribution of CWD to observed aboveground C pools was negligible, being always <1%. Across diversity levels and years, the CWD C pool ranged between 0.008 Mg ha^{-1} for six-species mixtures in 2006 to 0.423 Mg ha^{-1} for triplets in 2007. The CWD C varied markedly among plots. In one of the monoculture plots established with *Luehea seemanii,* the CWD C pool (0.546 Mg C ha^{-1}) was an order of magnitude greater than the mean of all monoculture plots (0.112 Mg C ha^{-1}). The pool of C in trees in that particular plot was likewise greater than the mean trees C pool of monocultures (26.4 Mg C ha^{-1} vs 13.5 Mg C ha^{-1}, respectively). MANOVA was used to test the effect of diversity on the allocation of C within the different aboveground pools, but no significant effect

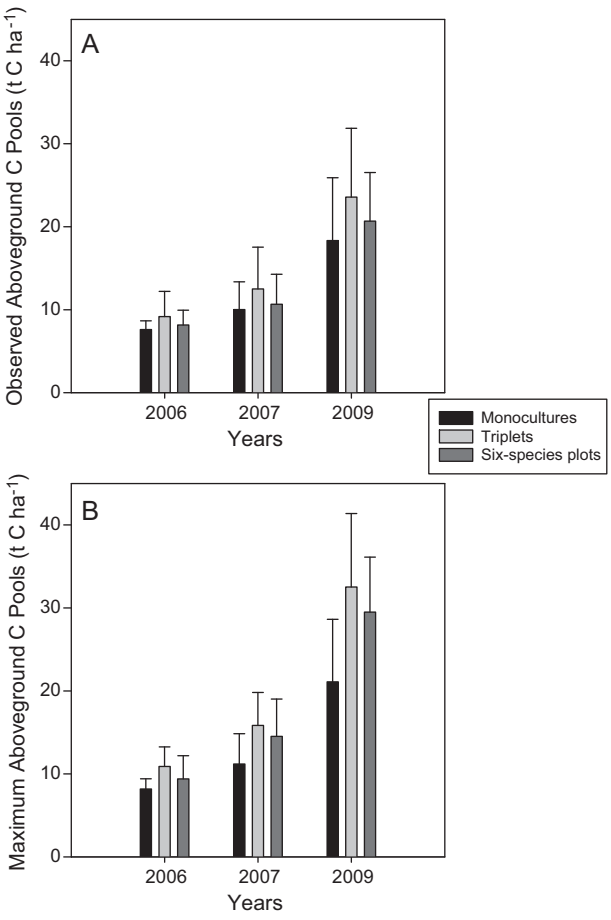

Fig. 1. Observed (A) and maximum (B) aboveground C pools for the three diversity levels for 2006, 2007 and 2009. Data are mean for each diversity level in year with standard deviation.

Table 2
Estimated contribution (%) of the four C pools measured to estimate observed aboveground C pools (OACP) and maximum aboveground C pool (MACP), controlling for tree mortality. Data are the mean in 2009 for each of the diversity levels with standard deviation in parentheses. CWD, coarse woody debris.

	Monocultures	Triplets	Six-species
OACS			
Trees	68.8 (14.2)	75.0 (8.9)	74.3 (5.3)
Herbaceous	29.2 (14.0)	22.7 (9.7)	24.0 (5.7)
Litter	1.4 (0.8)	1.6 (0.7)	1.0 (0.5)
CWD	0.5 (0.5)	0.8 (0.2)	0.7 (0.2)
MACS			
Trees	73.5 (11.9)	82.9 (5.3)	82.7 (2.8)
Herbaceous	24.7 (11.9)	15.6 (5.3)	16.4 (2.8)
Litter	1.2 (0.4)	0.6 (0.01)	0.7 (0.1)
CWD	0.6 (0.4)	0.8 (0.2)	0.8 (0.2)

Table 3
Results from analysis of variance comparing topsoil characteristics (0–10 cm) in 2001 and 2009. SOC, soil organic C concentration; SOCP, soil organic C pool. Significant effects are shown in italics.

Traits	Sources of variation	*F*	df	*p*
SOC	Year	*51.97*	*1*	*<0.0001*
	Diversity	0.96	2	0.96
	Year × diversity	2.54	2	0.093
$\delta^{13}C$	Year	*26.23*	*1*	*<0.001*
	Diversity	0.44	2	0.64
	Year × diversity	1.51	2	0.23
SOCP	Year	*52.12*	*1*	*<0.001*
	Diversity	1.71	2	0.11
	Year × diversity	1.40	2	0.38

was detected. The CWD C pool was stable through time, while the herbaceous C pool increased slightly. The fastest increment in C pool was for trees.

Maximum aboveground C pools, obtained by controlling for tree density, were also estimated (Fig. 1). As for observed aboveground C pools, the maximum aboveground C pools increased significantly through time ($F_{2,38} = 156.1$, $p < 0.0001$). The effects of both diversity and year × diversity on maximum aboveground C pools were also statistically significant ($F_{2,19} = 4.47$, $p = 0.025$ and $F_{4,38} = 398$, $p = 0.0085$ respectively). Maximum aboveground C pools for monocultures was 13.5 Mg C ha^{-1} compared with 19.8 Mg C ha^{-1} for triplets, a 46% difference. Through time, maximum aboveground C pools increased significantly less in monocultures than in mixture plots (Fig. 1). Trees C pool was a more important component of maximum aboveground C pool in mixtures than in monocultures, while herbaceous and litter C pools were more important components of maximum aboveground C pools in monocultures than in mixtures (Table 2). Not surprisingly the observed aboveground C pools were always smaller than maximum aboveground C pools.

Analysis of the RGR of aboveground C pools unveiled a marginally significant diversity effect on maximum aboveground C pools ($F_{2,21} = 2.81$, $p = 0.085$) with higher RGR at higher in triplets (36.3%) than in monocultures (30.1%) and six-species (28.8%), but no effect of diversity on observed aboveground C pools.

3.1.2. Soil properties

We observed strong changes in the concentration (SOC), pool (SOCP) and stable carbon isotope ratio ($\delta^{13}C$) of topsoil C during the establishment phase of the plantation (Fig. 2). Soil C decreased overall by 28.7% between 2001 and 2009. The reduction of SOC was strongest in the triplets (−40%), followed by monocultures (−25%) and the six-species mixtures (−19%). Similarly, SOCP decreased significantly from a mean of 34.5 ± 2.4 Mg C ha^{-1} in 2001 to 25.7 ± 5.7 Mg C ha^{-1} in 2009 (Table 3). The $\delta^{13}C$ decreased from −16.9‰ in 2001 to −20.8‰ in 2009. In 2001, about 80% of the organic matter in the soil was derived from C_4 pasture vegetation, whereas by 2009 this contribution had decreased to 49%, indicating increased inputs of organic matter by trees. On average, this means that the 2001 soil contained 27.6 Mg C ha^{-1} of C_4-derived C, which decreased to 12.6 Mg C ha^{-1} in 2009, whereas the C_3-derived C component increased from 6.9 to 13.1 Mg C ha^{-1}. These changes indicate the speed with which soil organic C turns over in this system. None of the soil characteristics that we measured showed a significant effect of diversity, but SOC responded marginally to the interaction between year and diversity (Table 3).

3.2. Carbon fluxes

3.2.1. Coarse woody debris decomposition

Besides changing through time, monthly CWD decomposition, measured as the proportional loss in CWD biomass, varied significantly as function of diversity, species and their interaction (Table 4). Monthly decomposition was significantly faster in monocultures, with a mean of 35 ± 24.1% compared with 31.3 ± 21.0% and 31.9 ± 26.8% for triplets and six-species mixtures, respectively. Across species, wood decomposition was slowest for *Luhea seemanii* (27.7 ± 26.5%) and highest for *Hura crepitans* (42.1 ± 21.8%), the other species having intermediate values of 29.1 ± 22.3% (*Tabebuia rosea*), 32.1 ± 14.5% (*Cedrela odorata*) and 33.6 ± 30.8% (*Anacardium excelsum*). After one month in the field, the woody stakes had lost, on average, between 19% (in six-species mixtures) and 29% (in monocultures) of the initial mass. It took six months to lose up to 40% of the initial mass and, after 10 months, the overall loss in biomass was 48 ± 20.5%. As time passed, the water content of the stakes increased to ~60%. Of the 600 stakes monitored in the experiment, only 21 stakes fully decomposed: nine were Hc, six Co, five Tr and one was Ae. The decay rate, *k*, did not differ significantly

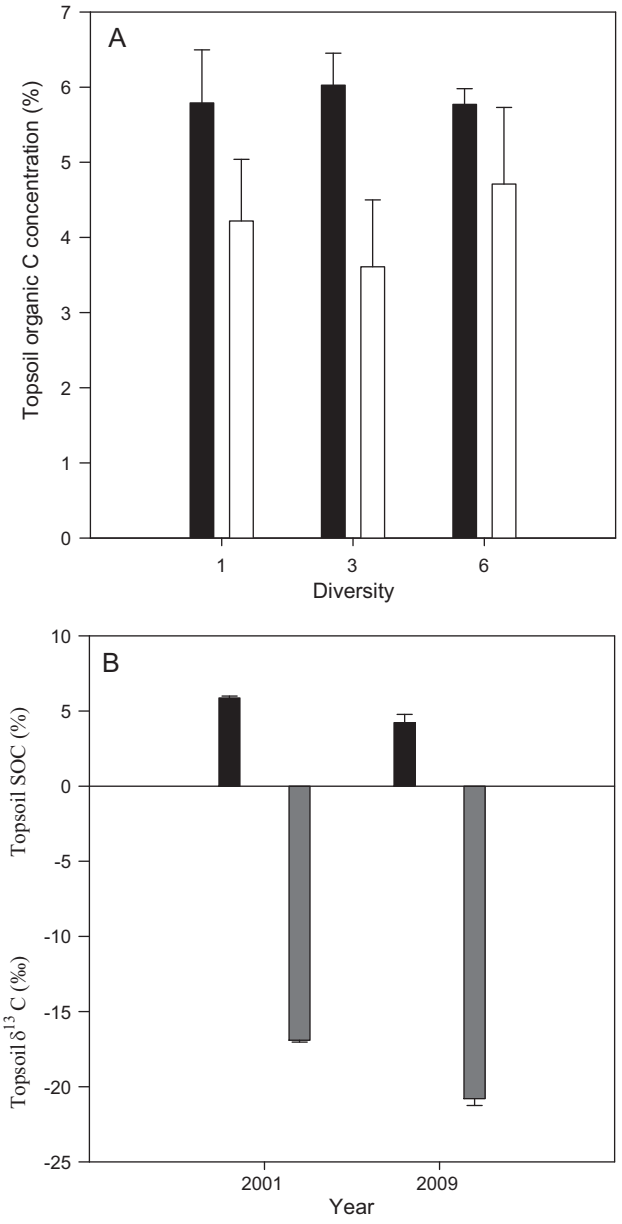

Fig. 2. (A) Comparison of topsoil (0–10 cm) soil organic C concentration (SOC) for the three diversity levels in 2001 and in 2009. The filled bars are data from 2001 and the empty bars are for 2009; (B) Mean value, across all diversity treatment for SOC and $\delta^{13}C$ values in 2001 and 2009. The black bars denote SOC and the grey bars are $\delta^{13}C$ values.

Table 4
Results of the ANOVA for the decomposition of coarse woody debris (CWD) between May 2007 and March 2008. Note that because data were replicated within a plot, the effect of diversity was tested against the appropriate error term plot (diversity). Significant effects are in italics.

Sources	df	SS	F	p
Diversity	*2*	*0.20*	*15.93*	*0.025*
Species	*4*	*1.36*	*15.92*	*0.0001*
Div × sp	*8*	*0.85*	*4.99*	*0.0001*
Month	*9*	*14.84*	*77.05*	*0.0001*
Div × month	18	0.57	1.48	0.09
Sp × month	*36*	*1.94*	*2.53*	*0.0001*
Div × sp × month	*71*	*2.36*	*1.56*	*0.0048*
Plot (div)	3	0.01	0.30	ns
Error	393	8.41		

among diversity levels, but responded significantly to the effect of species (diversity) ($F_{12,59} = 22.03$, $p < 0.0001$). Decay rate, k, ranged between -3.22 ± 0.39 for *Hura crepitans* grown in monocultures to -0.85 ± 0.20 for *Anacardium excelsum* in monocultures.

3.2.2. Litter decomposition

Data on litter decomposition were published previously (Scherer-Lorenzen et al., 2007). In brief, diversity had no significant effect on decomposition of the entire litter mixture (i.e., mixing species resulted in purely additive effects), although within mixtures, individual species decomposed at different rates depending on litter diversity. These results also highlighted the importance of species-specific effects on ecosystem processes.

3.2.3. Soil respiration

Soil respiration rates measured between pairs of trees were scaled to the plot level in the diversity plots to compare plot-level diversity effects. Using average respiration values from the monoculture pairs (model 1), respiration at the plot level was estimated to be $0.99 \pm 0.09\ \mu mol\ CO_2\ m^{-2}\ s^{-1}$. When scaling up using respiration of the mixed-species pairs (model 2), plot respiration was estimated to be $0.84 \pm 0.08\ \mu mol\ CO_2\ m^{-2}\ s^{-1}$. Our scaling-up calculations show that, at the plot level, there was a significant difference between the purely additive model and the model considering neighboring species interactions ($t = 15.35$, df = 11, $p < 0.001$), using the combined dry and early wet season data. This pattern was consistent across plots (Fig. 3).

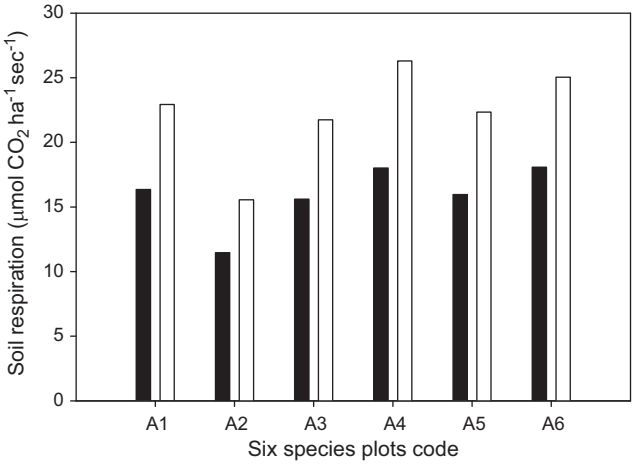

Fig. 3. Plot level soil respiration for each of the six-species mixtures using two scaling procedures. Empty bars represent plot respiration using monoculture scaling and filled ones using multipair scaling. For details see Murphy et al. (2008). Data used to estimate soil respiration at the plot level were obtained in March (dry season) and June (early wet season) of 2004.

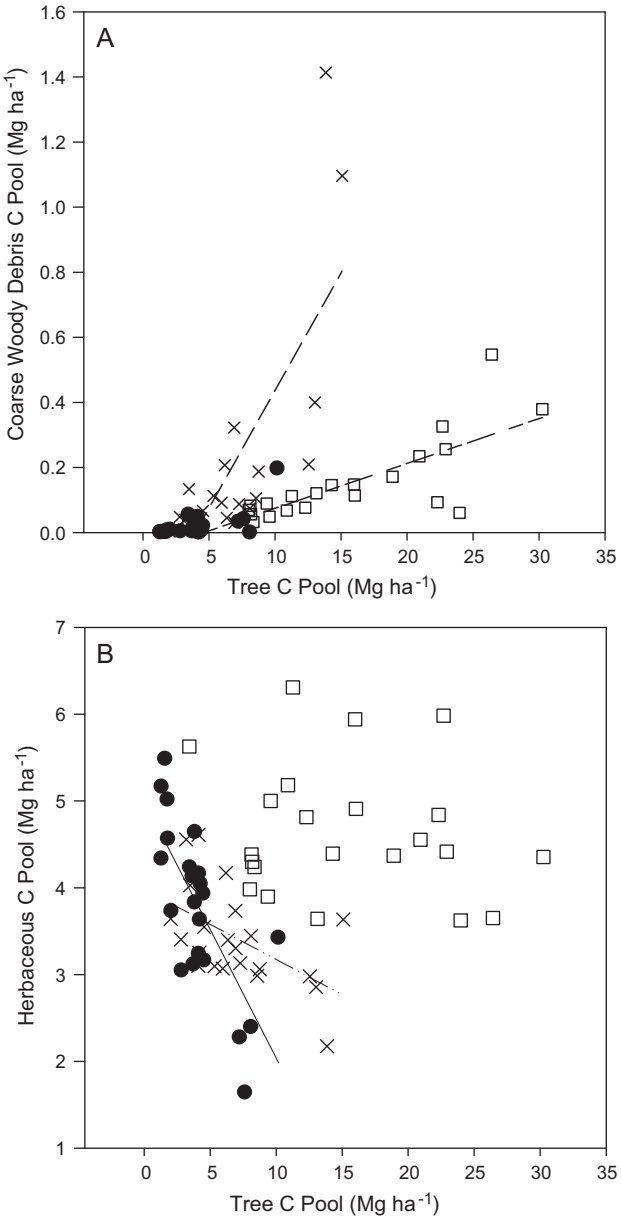

Fig. 4. Correlation between (A) the herbaceous vegetation and (B) coarse woody debris C pools and the trees C pools in 2006, 2007 and 2009. Symbols are: (●) 2006; (X) 2007; (□) 2009.

3.3. The ecosystem perspective

The relationship among the different components of the aboveground C pools was examined by correlation analyses, which revealed that the CWD C pool was significantly and positively correlated with the tree C pool in 2007 and 2009, but not in 2006 (2006: $r = 0.397$, $p = 0.225$; 2007: $r = 0.840$, $p = 0.001$; 2009: $r = 0.694$, $p = 0.017$). For the herbaceous C pool, we observed a significant negative correlation with the tree C pool in both 2006 and 2007 ($r = -0.724$, $p < 0.0001$, and $r = -0.539$, $p < 0.05$ respectively), but not in 2009 ($r = -0.168$, $p = 0.45$) (Fig. 4).

At the ecosystem level, three of the four aboveground C pools that were measured (trees, herbaceous vegetation and CWD) had their highest values in triplets and their lowest values in monocultures (Table 5). This pattern was also observed for litter production in the dry season of 2005 (Scherer-Lorenzen et al., 2007). Our results also indicate higher fluxes in monocultures than in mix-

Table 5
Magnitude and direction of diversity effects on observed C pools (OACP) and fluxes in the Sardinilla plantation. For trees, herbaceous and coarse woody debris (CWD), we compared mean C pools in 2009. For litter production, the difference was for our yearly estimate. Litter decomposition data are from Scherer-Lorenzen et al. (2007). Soil respiration data are from 2004 and for soil we calculated the difference in the loss of SOC between 2001 and 2009. Variables for which either the diversity or the time by diversity effects were statistically significant are in italics.

OACP components		Fluxes		Soil	
Tree	Triplets > monocultures +35%	Litter decomposition	Monocultures > triplets +5%	*Loss in SOC*	Triplets > monocultures +15.1%
Herbaceous	Triplets > monocultures +5%	*CWD decomposition*	Monocultures > triplets +11.8%		
Litter	Triplets > monocultures +7%	*Soil respiration*	Monocultures > mixture +17%		
CWD	Triplets > monocultures +73%				

tures, both for CWD decomposition and soil respiration. Finally, while the entire system lost SOC through time, the loss was most important in triplets and least important in six-species mixtures (Table 5).

Eight years after planting, the ecosystem C storage in the plantation, including tree roots and mineral topsoil C, ranged between 50.2 ± 11.4 Mg C ha^{-1} for monocultures and 54.5 ± 8.7 Mg C ha^{-1} for six-species plots (Fig. 5), triplets showing an intermediate value (51.8 ± 8.1 Mg C ha^{-1}). Assuming that the biomass C pool in 2001 was negligible (<1 Mg ha^{-1}), then the plantation gained in C, on average, ~20 and lost ~9 Mg ha^{-1} in biomass and soil respectively, for an overall gain of ~11 Mg ha^{-1} over 8 years. The plantation gained C during this period, with a mean accumulation of 16.1 ± 10.6 Mg C ha^{-1} in monocultures, 17.4 ± 9.5 Mg C ha^{-1} in triplets and 19.2 ± 8.6 Mg C ha^{-1} in six-species mixtures. Differences among the diversity levels were not statistically significant for either ecosystem C storage or gain. The two plots that gained most C between 2001 and 2009 were a six-species plot (35.3 Mg C ha^{-1}) and a triplet established with *Luhea seemanii*, *Anacardium excelsum* and *Tabebuia rosea* (32.9 Mg C ha^{-1}) (Fig. 5). Three monocultures also showed high C gains between 2001 and 2009: two were planted with *Tabebuia rosea* (31.2 Mg C ha^{-1} and 27.3 Mg C ha^{-1}) and the third one with *Cedrela odorata* (26.5 Mg C ha^{-1}). The plots with the lowest ecosystem C gain were the monocultures of *Hura crepitans* (1.3 and 1.8 Mg C ha^{-1}), in which the increments in C pools aboveground (12.8 and 9.3 Mg C ha^{-1}, respectively) were cancelled by losses of soil C of 13.9 and 8.4 Mg C ha^{-1}, respectively. Interestingly, *Hura* also had the highest leaf litter decomposition rate, presumably due to its low ratios of C:N and lignin:N (Scherer-Lorenzen et al., 2007).

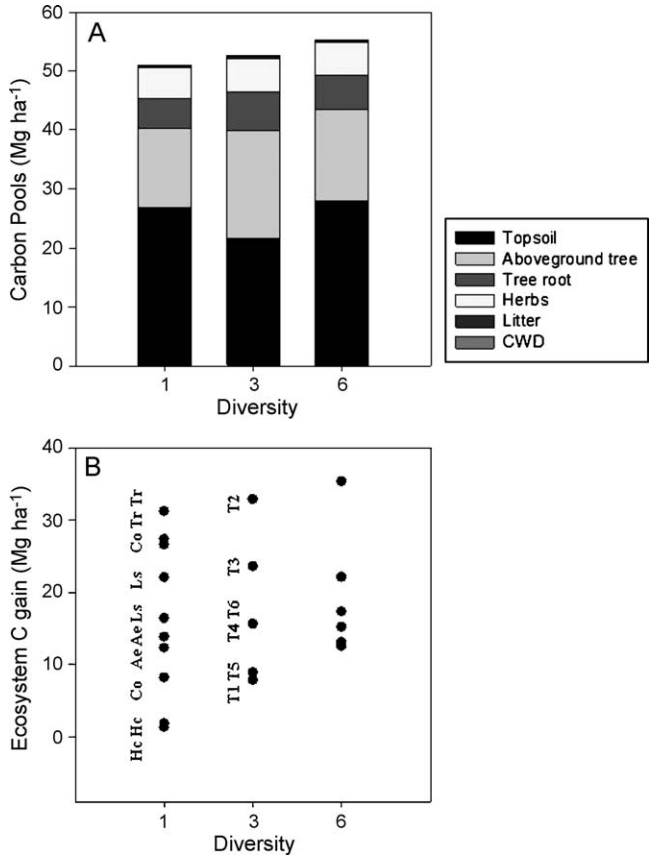

Fig. 5. (A) Total ecosystem C storage broken down in its different above- and belowground components in 2009 and (B) ecosystem C gain between 2001 and 2009 for each of the three different diversity levels. In (A) data are the mean of all plots established with the same number of species while, for (B), each circle represents an individual plot. Species planted in monocultures are abbreviated as follows: Ae, *Anacardium excelsum*, Co, *Cedrela odorata*, Hc, *Hura crepitans*, Ls, *Luehea seemanii*, Tr, *Tabebuia rosea*. The triplets are abbreviated as T1–T6 and were established with T1: *Co-Hc* and *Cordia alliodora (Ca)* that massively died after planting, T2: Ae-Ls-Tr, T3: Co-Ls-Ae, T4: Co-Hc-Ls, T5: Hc-Tr-Ca and T6: Ae-Tr-Ca.

We used path analyses to obtain a better understanding of the ecosystem response to diversity. We tested three models (Eq. (15)–(17)) to relate either SOC in 2009 or changes in SOC between 2001 and 2009, with four main aboveground C pools (trees, herbs, litter and CWD). The path model described in Eq. (15) had the highest explanatory power, with adjusted r^2 values of 0.136, 0.532 and 0.996 for monocultures, triplets and six-species mixtures, respectively. In monocultures, none of the paths were statistically significant, while two and four paths were statistically significant for triplets and six-species mixtures, respectively (Fig. 6). This suggests that, as diversity increases, the cycling of C is more tightly coupled among the different components of the ecosystem. In six-species mixtures, tree C is positively related to both CWD and litter production. Litter production, in turn, is positively correlated with SOC.

4. Discussion

4.1. Planting for carbon: an ecosystem approach

Eight years after reforestation in Sardinilla, the aboveground tree C pool and the topsoil organic C pool were found to be highly dynamic. We observed an increase in the size of the trees C pool concurrent with a decrease in the size of the soil C pool in the top 10 cm. As stated by other researchers (Catovsky et al., 2002; Körner, 2000), but often ignored, our results show that NPP, estimated from aboveground biomass, is not a good index of ecosystem C storage. The decreasing size of the topsoil C pool during the initial years following reforestation in Sardinilla shows that inputs to the topsoil C pool were smaller than the outputs. In the plantation, inputs to the soil are derived mainly from fine roots, litter production (Valverde-Barrantes, 2007) and CWD (Kirby and Potvin, 2007), while C is most probably lost through the decomposition of the previous grassland species and their root biomass. The observed changes in $\delta^{13}C$ provide some information on the source of the organic matter in the soil, showing clearly a changing contribution from C_4 pasture to C_3 trees. By 2009, about half of the soil organic matter in the topsoil was derived from trees, and this fraction is likely to increase with progressing plantation establishment. After plantation establishment in Sardinilla, the topsoil continued

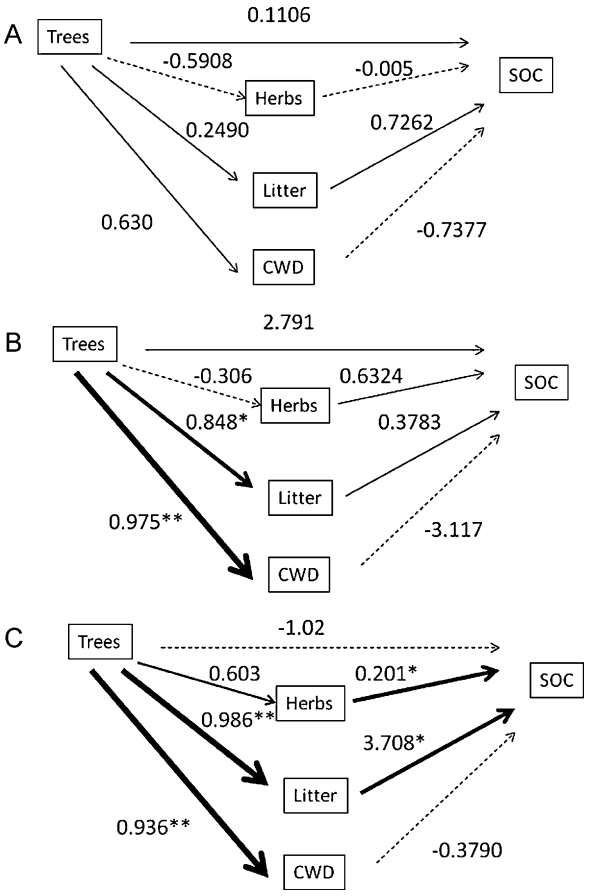

Fig. 6. Solved path diagram relating aboveground C pools with SOC for (A) monocultures, (B) triplets and (C) six-species mixtures. Solid lines represent positive relationships and dashed ones negative effects. Increasing width of the lines denotes statistically significant paths. The numbers are the path coefficients for each steps of the model. Path significance: $^{*}p < 0.05$ and $^{**}p < 0.01$.

to lose pasture SOC, which has not been replaced by plantation SOC. It should be noted that the decline in soil carbon is likely to be an underestimate, because we did not account for C in deeper soil horizons. These can contain a considerable proportion of the total soil C. In 2001, for example, seven soil profiles to a depth of 1 m contained 100–120 Mg C ha^{-1}, so that between 25% and 30% of the 1 m-deep SOC is contained in the top 10 cm (Abraham, 2004). In nearby Barro Colorado Island, for example, only 25% of the total soil C down to 1 m depth is found in the upper 10 cm, falling to 15% if 3 m depth is considered (B.L. Turner, unpublished data). Despite this, the biggest changes following perturbation can be expected in the topsoil as deeper soil carbon pools are considered fairly stable (Malhi and Davidson, 2009). We therefore assume that the majority of the changes following afforestation are captured in our dataset.

In the early years of plantation establishment a large proportion of tree productivity is allocated to developing storage organs (trunks and roots), rather than to yearly tissues such as leaves, thus reducing C flux to the soil C (Nair et al., 2009b; Yang et al., 2007). The magnitude and direction of changes in the soil C pool following reforestation can be highly variable and dependent on a variety of factors, including previous land use, species and soil properties (Epron et al., 2009). Meta-analysis have indicated a 10% reduction of soil C pools with land conversion from pasture to plantation (Guo and Gifford, 2002) and reductions in soil C between 6.7% and 15% across 153 sites with diverse plantation types (Berthrong et al., 2009). Both studies indicated that *Pinus* plantations in particular reduced soil N due to rapid plant uptake. In comparison, broadleaf tree plantations reduced soil C pools (Guo and Gifford, 2002). Don et al. (2009) reported a significant C loss (41% reduced gross primary production) only during the first year after conversion from pasture to tree plantation due to site preparation, including ploughing. In Sardinilla, however, the soil was not ploughed before planting. Standard reforestation practice in Panama is to hand clean the field and open a hole, the size of seedling's roots, thus minimizing ecosystem disruption.

The precipitation regime might provide a possible explanation for the observed loss of SOC during reforestation in Sardinilla. Guo and Gifford (2002) reported that plantations established in areas with >1500 mm yr^{-1} rainfall showed an average −23% decreases in SOC. Precipitation at the nearby meteorological station of Salamanca, about 5 km to the northeast of the Sardinilla plantation, has a long term mean (1972–2009) of 2289 mm, raising the possibility that an important amount of C might be lost from the site after heavy rains. Net ecosystem production that ultimately determines C storage has been described as the balance between autotrophic C uptake by photosynthesis and respiration (Catovsky et al., 2002), although in the humid tropics C loss via runoff as dissolved organic C must also be accounted for (Schwendenmann and Veldkamp, 2005; Goller et al., 2006; Fujii et al., 2009).

Root biomass and dynamics are important unknown in global C pools (Nair et al., 2009a). Robinson (2007) suggested that available data on root biomass underestimate their contribution to the total biomass by 40%. We used site- and species-specific allometric relationships to estimate root biomass and, therefore, the root C pool, although this approach suffered from several shortcomings. First, we assumed that the root/shoot ratio remained identical as trees grew and that these ratios were also not sensitive to neighborhood-effects, although these assumptions that were not tested. Furthermore, due to the clay-rich nature of the soil, we were unable to sample and weigh fine roots (Coll et al., 2008). According to Nair et al. (2009a) fine roots could represent as much as 33% of global NPP, although high turnover of fine roots imply that this part of NPP will not strongly affect long-term C sequestration into the soil C pool. The root/shoot ratios that we used to estimate the root C pool did not account for fine roots, thus underestimating the true size of this pool.

In 2009, the aboveground tree C pool in Sardinilla ranged between 30.2 Mg C ha^{-1} for a triplet established with Ae, Ls and Tr and 3.4 Mg C ha^{-1} for a monoculture plot of Hc, with a mean value of 15.7 Mg C ha^{-1}. These values compare well with data from a 9–14-year-old native tree plantation in Costa Rica that reported a range of 12.4–79.1 Mg C ha^{-1} (Redondo-Brenes, 2007) and are in line with an early report of C pools in the aboveground plant component of tropical plantations (Schroeder, 1992). Overall, despite the reduction in the size of the topsoil C pool, the Sardinilla plantation has acted as a net C sink since its establishment, with an average of 18.39 Mg C ha^{-1} gained over 8 years. Further, the observed exponential tree growth suggests that the sink strength will increase through time, at least in the medium term. Our results show clearly that the aboveground tree C pool represents only half of the observed ecosystem C storage with the mineral topsoil (not including soil carbon > 10 cm deep), with herbs and tree roots being other important C pools.

4.2. Biodiversity and C pools

The possibility that mixed-species plantations might increase ecosystem C storage has been often cited as a reason for the promotion of reforestation with native species (Diaz et al., 2009; Piotto et al., 2010; Caspersen and Pacala, 2001). A variety of hypotheses have been proposed to explain the expected relationship between tree diversity and ecosystem C storage. Diaz et al. (2009), along with Catovsky et al. (2002), stated that biodiversity could affect (1) the rates of C gain or loss, (2) the size of C pool, and (3) temporal sta-

bility and hence the lifespan or stability of C pools. In other words, if biodiversity influences either the size of C pools or fluxes, it will affect ecosystem C storage. However, in Sardinilla, the strongest diversity effects that we uncovered were on the links between the different pools of C, rather than on the pools themselves. The C pools, whether ecosystem or aboveground only, did not respond significantly to diversity.

Results from other mixed-species plantations suggest that the identity of the dominant species plays an important role in determining C gained by the trees (Redondo-Brenes, 2007; Valverde-Barrantes, 2007). For example Piotto et al. (2010) found positive biodiversity effects in one of three 15–16-year-old native plantations and showed that, in economical terms, mixed species plantations outperformed monocultures. Ewel and Mazzarino (2008) suggested that traits such as leaf phenology could explain the outcome of mixed species plantations and argued that no unique outcome of planting mixed species was to be expected. In Sardinilla, mixtures forming a stratified canopy (e.g. with the fast-growing Ls in the upper canopy, the moderately growing Ae in the middle canopy, and the late successional and slow-growing species Tr in the lower canopy) are especially promising. Such stratification is often mentioned as a prerequisite for complementary resource use in mixed stands, leading to higher productivity (Kelty, 1992; Scherer-Lorenzen et al., 2005a). Furthermore, the inclusion of species with rather high limits of litter decomposition (Berg and McClaugherty, 2003), such as those with recalcitrant litter, would also allow for sustained accumulation of humus and hence C in the soil. Data from litter production and decomposition from our plantation suggest that admixing Ls and Ae would also contribute to C sequestration, due to their high rates of litter and CWD production, combined with rather low decomposition rates and low N concentrations in litter (Scherer-Lorenzen et al., 2007).

It has been noted elsewhere that tree mortality plays an important role in determining the biomass of a plantation (Vila et al., 2007; Paquette and Messier, 2010). Mortality was species-specific in Sardinilla, and we provided strong evidence for species complementarity when controlling for tree mortality (Potvin and Gotelli, 2008). Following these results we chose to report aboveground C pools as either observed or maximum values. To calculate observed aboveground C pools, the data was scaled up to a standard area, chosen as one hectare, to correct for different plot sizes. The second estimate of aboveground C pools assumed no mortality and used the average biomass value of living trees of each species to estimate plot-level tree biomass. Maximum aboveground C pools, controlling for tree density and associated RGR, were shown to respond significantly to diversity, yet observed aboveground C pool did not. Mortality apparently obscured the diversity effect as reported elsewhere (Erskine et al., 2006; Petit and Montagnini, 2006), despite the possibility that dead trees might free resources allowing living trees to grow bigger. The important work done by Ewel and collaborators in La Selva, Costa Rica, could provide an explanation (Haggar and Ewel, 1997; Ewel and Mazzarino, 2008). In 1991, they established monocultures and polycultures of three native tree species (*Hyeronima alchorneoides*, *Cedrela odorata* and *Cordia alliodora*) in association with *Euterpe oleracea* and *Heliconia imbricata* forming a sub-canopy cover. After 13 years of growth, their results show that deciduousness of the tree species is a key characteristic determining the productivity of the ecosystem. *Hyeronima*, being evergreen, was able to maintain a high aboveground net primary production, while the net aboveground production of *Cordia*, and to a lesser extent *Cedrela*, was severely limited by *Euterpe* and *Heliconia*. We suggest that tree mortality in Sardinilla stimulated the herbaceous layer rather than growth of the remaining living trees.

We nevertheless uncovered three significant or marginally significant effects of diversity or its interaction (CWD decomposition, soil respiration, and the reduction of SOC) and used path analyses to examine relationships between these different pools and fluxes. The results unequivocally showed that, as diversity increases, the links between the different C pools and fluxes become significantly stronger. The strongest diversity effects in the Sardinilla plantations were on the links between the different pools of C, rather than on the size of the pools themselves. Eight years after establishment of the tree plantation on a former pasture, tree diversity affected the processes governing the changes in C pools and fluxes related to the land-use change. We conclude that the choice of tree mixtures for afforestation in the tropics alters ecosystem C storage. Our ability to make stronger inferences from this finding is curtailed by the quality of some of the estimates that we used (e.g. for litter production) and because of the temporal variation in some of the data used (e.g. soil respiration data from 2004). Our results, however, strongly suggest that an integrated ecosystem approach, in which all ecosystem characteristics linked to both C pools and C cycling are included, is required to gain an accurate representation of the effect of biodiversity on this important ecosystem function. We are currently planning such an integrated effort in Sardinilla for the 10th year of the plantation in 2011.

Acknowledgements

The Sardinilla plantation was established with the help to a grant from the Ministère de la Recherche, de la Science et de la Technologie du Québec. Continuous support from the Smithsonian Tropical Research Institute was invaluable in our ability to maintain this site on the long term. C.P. acknowledges the support from a Discovery grant of the Natural Sciences and Engineering Council of Canada. Jose Luis Bonnilla collected litter fall and analyzed the litter decomposition bags in 2005. In 2007 and 2008 litter fall and the herbaceous vegetation were sampled by Simone Tapernon, Timo Mark, and Lisa Werther, while Volker Hahn helped in measuring soil respiration. This research was funded by the German Research Foundation (DFG Wi 1601/6-1) which we gratefully acknowledge. N.B. and S.W. appreciate funding from the North-South Centre (former Swiss Centre for International Agriculture) of ETH Zurich, Switzerland. Financial support was provided to M.S.-L. and Jan Jansa (ETH Zurich) by the Swiss National Science Foundation (3100A0-110031/1).

References

Abraham, M., 2004. Spatial variation in soil organic carbon and stable carbon isotope signature in a pasture and a primary forest in Central Panama. M.Sc. thesis, Department of Geography, McGill University. 91 pp.

Berthrong, S.T., Jobbágy, E.G., Jackson, R.B., 2009. A global meta-analysis of soil exchangeable cations, pH, carbon, and nitrogen with afforestation. Ecological Applications 19, 2228–2241.

Caspersen, J.P., Pacala, S.W., 2001. Successional diversity and forest ecosystem function. Ecological Research 16, 895–903.

Catovsky, S., Bradford, M.A., Hector, A., 2002. Biodiversity and ecosystem productivity: implications for carbon storage. Oikos 97, 443–448.

Coll, L., Potvin, C., Messier, C., Delagrange, S., 2008. Carbon storage, allocation patterns and root architecture of tropical trees with contrasting shade tolerance in young mixed plantations. Trees—Structure and Function 22, 585–596.

Delagrange, S., Coll, L., Messier, Ch., Potvin, C., 2008. Linking multiple-level tree traits with biomass accumulation in native tree species used for reforestation in Panama. Trees—Structure and Function 22, 337–349.

Diaz, S., Hector, A., Wardle, D.A., 2009. Biodiversity in forest carbon sequestration initiatives: not just a side benefit. Current Opinion in Environmental Sustainability 1, 55–60.

Don, A., Rebmann, C., Kolle, O., Scherer-Lorenzen, M., Schulze, E.D., 2009. Impact of afforestation-associated management changes on the carbon balance of grassland. Global Change Biology 15, 1990–2002.

Elias, M., Potvin, C., 2003. Assessing intra- and inter-specific variation in trunk carbon concentration for 32 neotropical tree species. Canadian Journal of Forest Research 33, 1039–1045.

Epron, D., Marsden, C., M'Bou, A.T., Saint-André, L., d'Annunzio, R., Nouvellon, Y., 2009. Soil carbon dynamics following afforestation of a tropical savannah with Eucalyptus in Congo. Plant and Soil 323, 309–322.
Erskine, P.D., Lamb, D., Bristow, M., 2006. Tree species diversity and ecosystem function: can tropical mixed-species plantations generate greater productivity? Forest Ecology and Management 233, 205–210.
Ewel, J.J., Mazzarino, M.J., 2008. Competition from below for light and nutrients shifts productivity among tropical species. Proceedings of the National Academy of Sciences (USA) 105, 18836–18841.
Firn, J., Erskine, P.D., Lamb, D., 2007. Woody species diversity influences productivity and soil nutrient availability in tropical plantations. Oecologia 154, 521–533.
Fujii, K., Uemura, M., Hayakawa, C., Funakawa, S., Sukartiningsih, Kosaki, T., Ohta, S., 2009. Fluxes of dissolved organic carbon in two tropical forest ecosystems of East Kalimantan, Indonesia. Geoderma 152, 127–136.
Goller, R., Wilcke, W., Fleischbein, K., et al., 2006. Dissolved nitrogen, phosphorus, and sulfur forms in the ecosystem fluxes of a montane forest in Ecuador. Biogeochemistry 77, 57–89.
Guo, L.B., Gifford, R.M., 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology 8, 345–360.
Haggar, J.P., Ewel, J.J., 1997. Primary productivity and resource partitioning in model tropical ecosystems. Ecology 78, 1211–1221.
Harmon, M.E., Sexton, J., 1996. Guidelines for Measurements of Woody Detritus in Forest Ecosystem LTER Network Office. University of Washington, Seattle, WA.
Kelty, M.J., 1992. Comparative productivity of monocultures and mixed-species stands. In: Kelty, M.J., Larson, B.C., Oliver, C.D. (Eds.), The Ecology and Ssilviculture of Mixed-species Forests. Kluwer Academic Publishers, Dordrecht/Boston/London, pp. 125–141.
Kirby, K.R., Potvin, C., 2007. Species-level management in carbon sink projects: a case study from an Embera territory in eastern Panama. Forest Ecology and Management 246, 208–221.
Körner, Ch., 2000. Biosphere responses to CO_2 enrichment. Ecological Applications 10, 1590–1619.
Körner, C., 2003. Slow in, rapid out—carbon flux studies and Kyoto targets. Science 300, 1242–1243.
Malhi, Y., Baldocchi, D.D., Jarvis, P.G., 1999. The carbon balance of tropical, temperate and boreal forests. Plant Cell and Environment 22, 715–740.
Malhi, Y., Davidson, E.A., 2009. Biogeochemistry and ecology of terrestrial ecosystems in Amazonia. In: Keller, M., Bustamante, M., Gash, J., Dias, P.S. (Eds.), Amazonia and Global Change. American Geophysical Union, Washington, DC, pp. 293–298.
Malmer, A., Murdiyarso, D., Bruijnzeel, L.A., Ilstedt, U., 2010. Carbon sequestration in tropical forests and water, a critical look at the basis for commonly used generalizations. Global Change Biology 16, 599–604.
Murphy, M., Balser, T., Buchmann, N., Hahn, V., Potvin, C., 2008. Linking tree biodiversity to belowground process in a young tropical plantation: impacts on soil CO_2 flux. Forest Ecology and Management 255, 2577–2588.
Nair, P.K.R., Nair, V.D., Kumar, B., Haile, S.G., 2009a. Soil carbon sequestration in tropical agroforestry systems: a feasibility appraisal. Environmental Science and Policy 12, 1099–1111.
Nair, P.K.R., Kumar, B., Nair, V.D., 2009b. Agroforestry as a strategy for carbon sequestration. Journal of Plant Nutrition and Soil Science 172, 10–23.
Oelmann, Y., Potvin, C., Mark, T., Werther, L., Tapernon, S., Wilcke, W., 2010. Tree mixture effects on aboveground nutrient pools of trees in an experimental plantation in Panama. Plant and Soil 326, 199–212.
Paquette, A., Messier, C., 2010. The role of plantations in managing the world's forests in the Anthropocene. Frontiers in Ecology and the Environment 8, 27–34.
Petit, B., Montagnini, F., 2006. Growth equations and rotation ages of ten native tree species in mixed and pure plantations in the humid Neotropics. Forest Ecology and Management 199, 243–257.
Piotto, D., Craven, D., Montagnini, F., Alice, F., 2010. Silvicultural and economic aspects of pure and mixed native tree species plantations on degraded pasturelands in humid Costa Rica. New Forests 39, 369–385.
Potvin, C., Gotelli, N., 2008. Biodiversity enhances individual performance but does not affect survivorship in tropical trees. Ecology Letters 11, 217–223.
Redondo-Brenes, A., 2007. Growth, carbon sequestration, and management of native tree plantations in humid regions of Costa Rica. New Forests 34, 253–268.
Robinson, D., 2007. Implications of a large global root biomass for carbon sink estimates and for soil carbon dynamics. Proceedings of the Royal Society B 274, 2753–2759.
Scherer-Lorenzen, M., Bonilla, J.L., Potvin, C., 2007. Tree species richness affects litter production and decomposition dynamics in a tropical biodiversity experiment. Oikos 116, 2108–2124.
Scherer-Lorenzen, M., Körner, C., Schulze, E.-D., 2005a. The functional significance of forest diversity: a synthesis. In: Scherer-Lorenzen, M., Körner, C., Schulze, E.-D. (Eds.), Forest Diversity and Function: Temperate and Boreal Systems. Ecological Studies, vol. 176. Springer, Berlin, Heidelberg, New York, pp. 377–389.
Scherer-Lorenzen, M., Potvin, C., Koricheva, J., Schmid, B., Hector, A., Bornik, Z., Reynolds, G., Schulze, E.D., 2005b. The design of experimental tree plantations for functional biodiversity research. In: Scherer-Lorenzen, M., Kvrner, C., Schulze, E.-D. (Eds.), The Functional Significance of Forest Diversity. Ecological Studies, vol. 176. Springer, Heidelberg/New York, pp. 347–376.
Schwendenmann, L., Veldkamp, E., 2005. The role of dissolved organic carbon, dissolved organic nitrogen, and dissolved inorganic nitrogen in a tropical wet forest ecosystem. Ecosystems 8, 339–351.
Seitlinger, T., 2008. Einfluss der Artenzahl auf die Phosphorgehalte der oberirdischen Biomasse und auf die Phosphorverfügbarkeit im Boden. Master thesis, Department of Geography, University of Zurich. 58 pp.
Schlapfer, F., Schmid, B., 1999. Ecosystem effects of biodiversity: a classification of hypotheses and exploration of empirical results. Ecological Applications 9, 893–912.
Schlesinger, W.H., Lichter, J., 2001. Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO_2. Nature 411, 466–469.
Schroeder, P., 1992. Carbon storage potential of short rotation tropical tree plantations. Forest Ecology and Management 50, 31–41.
Schulze, E.-D., Wirth, C., Heimann, M., 2000. Climate change: managing forests after Kyoto. Science 289, 2058–2059.
Valverde-Barrantes, O.J., 2007. Relationships among litterfall, fine-root growth and soil respiration for five tropical tree species. Canadian Journal of Forestry Research 37, 1954–1965.
Vila, M., Vayareda, J., Comas, L., Ibanez, J.J., Mata, T., Obon, B., 2007. Species richness and wood production: a positive association in Mediterranean forests. Ecology Letters 10, 241–250.
Wishnie, M.H., Dent, D.H., Mariscal, E., Deago, J., Cedeno, N., Ibarra, D., Condit, R., Ashton, P.M.S., 2007. Initial performance and reforestation potential of 24 tropical tree species planted across a precipitation gradient in the Republic of Panama. Forest Ecology and Management 243, 39–49.
Yang, Y.-S., Chen, G.-S., Guo, J.-F., Xie, J.-S., Wang, X.-G., 2007. Soil respiration and carbon balance in a subtropical native forest and two managed plantations. Plant Ecology 193, 71–84.