



Evidence of old carbon used to grow new fine roots in a tropical forest

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Summary

- In this study, we explore how a hurricane disturbance influenced carbon allocation for the production of new fine roots.
- Before and after a hurricane, we measured the age of carbon (time since fixation from the atmosphere) in fine root structural tissues using natural abundance radiocarbon (¹⁴C) measured by accelerator mass spectrometry. Roots were sampled from five seasonally dry tropical forests ranging in age from 6 yr to a mature forest.
- Structural carbon in combined live + dead roots picked from soil cores sampled 1 month before the hurricane had mean ages ranging from 4 to 11 yr, whereas live roots alone had ages of 1–2 yr. Structural carbon in new live fine roots produced over a period lasting from 3 wk before the hurricane to 2 months after the event had mean ages of between 2 and 10 yr.
- Contrary to expectations, our results showed that plants allocate long-lived storage carbon pools to the production of new fine roots after canopy defoliation and root mortality. The age of the carbon allocated for new roots increased with forest age and forest above-ground biomass, suggesting an adaptation of plants to survive and recover from severe disturbances.

Introduction

How below-ground processes respond to changes in climatic conditions and large-scale disturbances is unclear, but is critical for the understanding of ecosystem carbon dynamics. Fine roots are an important carbon pool in tropical forests (Jaramillo *et al.*, 2003; Vargas *et al.*, 2008), and their production and turnover rates are key parameters for accurate estimates of net primary productivity (Vogt *et al.*, 1996; Clark *et al.*, 2001). Fine roots are important for water and nutrient uptake (Pregitzer, 2002), and they contribute to the autotrophic component of soil respiration (Hanson *et al.*, 2000; Silver *et al.*, 2005). In addition, fine roots form a symbiotic association with mycorrhizal fungi, which favors tree growth (Allen *et al.*, 2003), and produce exudates (e.g. glomalin) that contribute to a large proportion of longer lived carbon in tropical soils (Rillig *et al.*, 2001).

The understanding of fine root dynamics is challenging because of technical and methodological limitations (Guo *et al.*, 2008a; Pritchard & Strand, 2008; Strand *et al.*, 2008), but is a key parameter for the accurate assessment of ecosystem carbon (Norby *et al.*, 2000). Two critical questions for ecosystem carbon dynamics are:

- How do fine root dynamics respond to climatic variation?
- How do plants allocate resources to maintain fine roots under changing environmental conditions?

These questions are critical, because it is important to link plant carbon assimilation with root carbon utilization to improve the mechanistic understanding of plant–ecosystem carbon dynamics (Hogberg *et al.*, 2002; Guo *et al.*, 2004; Keel *et al.*, 2006). In this study, we addressed climatic variability in terms of a large-scale wind disturbance and the implications on carbon allocation for the production of new fine roots.

We used radiocarbon (¹⁴C) measurements of fine root tissues to study fine root dynamics in a tropical forest. Most studies describing root radiocarbon age have been limited to temperate forests (Gaudinski *et al.*, 2001; Tierney & Fahey, 2002; Joslin *et al.*, 2006; Vargas *et al.*, 2008), with the exception of two studies in tropical forests (Trumbore *et al.*, 2006; Vargas *et al.*, 2009). These studies are consistent in that the age of structural carbon in fine bulk (dead + live) roots averages from 3 to 18 yr. By contrast, newly grown fine roots are constructed from photosynthetic products of < 2 yr in age in temperate (Gaudinski *et al.*, 2001; Joslin *et al.*, 2006) and tropical (Trumbore *et al.*, 2006) forests. Furthermore, stable isotope (¹³C) approaches

have also confirmed that recently fixed photosynthetic products are used to produce new fine roots in temperate forests (Matamala *et al.*, 2003; Keel *et al.*, 2006).

Although previous studies have shown that recently assimilated carbon is used to produce new fine roots, it is possible that older carbon stored in nonstructural carbon (NSC) pools may be transferred for the production of these structures (Luo, 2003), especially in systems with frequent and severe disturbances (Langley *et al.*, 2002). Not accounting for the mobilization of older stored carbon to produce new fine roots could contribute to inaccurate estimates of fine root longevity and below-ground net primary productivity (Luo *et al.*, 2004), and has implications for our understanding of plant recovery and resilience adaptations (Langley *et al.*, 2002). However, little is known about the sizes, ages and ecological roles of NSC pools (Körner, 2003; Wurth *et al.*, 2005; Poorter & Kitajima, 2007), but the ability to store carbon in these pools may determine plant responses to climate change.

If plants have the ability to allocate stored carbon to produce new roots, when do they do this? One hypothesis is that, under stress conditions, caused by disturbances that reduce structural carbon pools, plants may have the ability to allocate stored carbon to rapidly replace structures. For example, plants may be damaged by herbivores, fire or wind (e.g. hurricanes), which will reduce the leaf area index and root biomass. In response, plants could initially invest carbon to grow new leaves for photosynthesis and new fine roots for water and nutrient uptake, but, to date, it is unclear whether plants allocate recently fixed carbon or older stored carbon.

In this study, we have taken advantage of a hurricane disturbance in a fire chronosequence of seasonally dry tropical forests (SDTFs) in the Yucatan Peninsula, Mexico (Vargas *et al.*, 2008). We assessed the mean age (time since fixation from the atmosphere) of carbon in fine root structural tissues using radiocarbon (^{14}C) in fine roots before and after hurricane Wilma in 2005. We sampled forests of different ages, ranging from 6 to > 60 yr (mature forest) since the last stand-killing fire. First, we studied the impact of hurricane disturbance on canopy openness and

total fine root biomass. Second, we measured the radiocarbon of live and bulk (dead + live) fine roots collected before the hurricane. Third, we had the unusual and unique opportunity of sampling new fine roots produced up to 2 months after the hurricane event by taking advantage of root in-growth cores installed just before the storm. We tested the following hypotheses: (1) canopy openness increases and root biomass decreases after the hurricane; (2) the mean radiocarbon age of live and bulk fine roots averages between 3 and 18 yr and may increase with forest age; (3) live roots are constructed from recent (< 2-yr-old) photosynthetic products and may not change with forest age; and (4) new roots produced after the hurricane are also constructed from recent (< 2-yr-old) photosynthetic products and may not change with forest age.

Materials and Methods

Study area

The study was conducted at El Eden Ecological Reserve, Quintana Roo, Mexico (latitude 21°12'N; longitude 87°11'W). The site is an SDTF with semi-deciduous vegetation and uniform soils and climate. The mean annual air temperature is 24.2°C and annual precipitation is 1650 mm (years 1998–2005), with most falling from June to December. The landscape is flat, with an elevation of 6 m above sea level. Soils are shallow (depth < 20 cm), with high organic matter content (nearly 30%), and underlain by limestone bedrock (Allen *et al.*, 2003; Vargas *et al.*, 2008). Severe fires at the study site occurred during the dry seasons of 1999, 1995, 1989 and 1975, affecting different areas of the Reserve and creating forest stands of different ages (Vargas *et al.*, 2008). All forest stands and mature forests are located within a distance of 8 km of each other. During 2005, the ages of regrowth for these forest stands were 6, 10, 16 and 30 yr, respectively. In addition, the Reserve has patches of mature forests with no evidence of fire for > 60 yr. Thus, the stands differ in above-ground biomass, forest structure (Table 1) and species' composition (Table S1, see Supporting Information).

Table 1 Forest structural characteristics (tree density, tree basal area, tree height) and above-ground biomass pools of five seasonally dry tropical forests in the Yucatan Peninsula, Mexico

Site age (years)	Tree category DBH 1–9.9 cm			Tree category DBH 10–29.9 cm			Tree category DBH >30 cm			
	Density (stems ha ⁻¹)	Basal area (m ² ha ⁻¹)	Height (m)	Density (stems ha ⁻¹)	Basal area (m ² ha ⁻¹)	Height (m)	Density (stems ha ⁻¹)	Basal area (m ² ha ⁻¹)	Height (m)	AGB (Mg ha ⁻¹)
6	24 950 (7045)	5 (1.5)	2.2 (0.1)	0	0	0	0	0	0	20.2 (2.1)
10	26 233 (2314)	12.8 (2.8)	4.0 (0.2)	25 (13)	0.3 (0.1)	7.5 (0.1)	0	0	0	37.2 (2.3)
16	31 833 (5296)	21.2 (3.4)	4.2 (0.1)	90 (42)	0.9 (0.5)	8.4 (0.1)	0	0	0	62.8 (2.8)
30	14 166 (4060)	12.2 (3.2)	4.5 (0.3)	502 (132)	8.0 (2.3)	9.6 (0.2)	0	0	0	77.4 (4.4)
MF	11 000 (2620)	12.1 (3.3)	5.7 (0.5)	669 (116)	13.9 (2.6)	10.6 (0.2)	38 (24)	19.6 (3.2)	13.3 (0.9)	143.9 (6.5)

Tree categories represent cohort of trees of a DBH range in a forest stand. AGB, above-ground biomass; DBH, tree diameter at breast height (1.3 m); MF, mature forest. Means and standard errors are from data collected by Vargas *et al.* (2008).

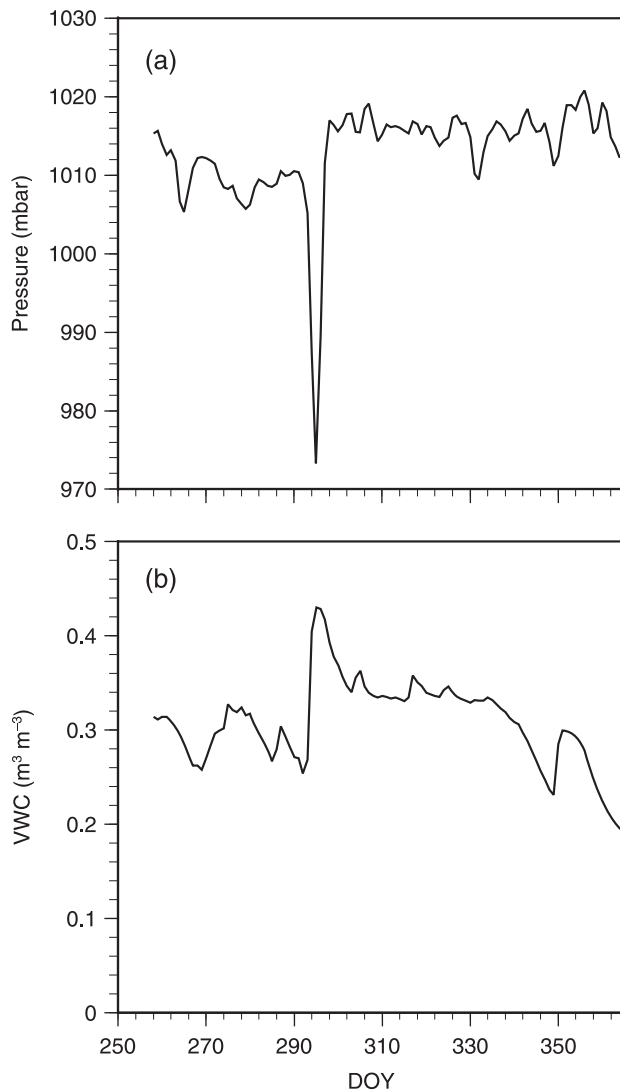


Fig. 1 Climatic variables of barometric pressure (a) and soil volumetric water content (VWC) (b) in a seasonally dry tropical forest, Mexico, between day of the year (DOY) 250 and 365 of 2005. Hurricane Wilma made landfall over the study site on October 22nd 2005 as a Category 4 storm on the Saffir–Simpson scale, and emerged over the Gulf of Mexico on October 23rd 2005 as a Category 2 storm.

Previous studies have described the Reserve (Gómez-Pompa *et al.*, 2003) and compiled detailed information about vascular species' composition at the study site (Schultz, 2005).

Hurricane Wilma

Hurricane Wilma, the most intense hurricane on record in the Atlantic basin, made landfall over the island of Cozumel, Mexico on October 21st 2005 as a Category 4 storm on the Saffir–Simpson scale. It emerged over the Gulf of Mexico on October 23rd as a Category 2 storm, crossing over the study site (Vargas & Allen, 2008a). Hurricane Wilma lowered the barometric pressure from nearly 1010 to 975 mbar (Fig. 1a) in *c.* 19 h on

October 22nd 2005 when the eye of the storm crossed over the study site. We estimated that the barometric pressure was < 1000 mbar for a total of 40 h with sustained winds between 170 and 190 km h⁻¹ according to the National Oceanic and Atmospheric Administration National Hurricane Center (www.nhc.noaa.gov). Soil volumetric water content was increased by the intense rains and remained above 0.3 m³ m⁻³ for nearly 50 d following the storm before the water table decreased (Fig. 1b).

Canopy openness

During September 2005, before the hurricane, we established three 60-m transects within each forest, including a mature forest. The distance separating each transect was nearly 100 m. Hemispherical photographs were taken below the forest canopy during the last week of September 2005 at three randomly selected sampling points along each transect. Percentage canopy openness was calculated using a Gap Light Analyzer V2.0 with the 60° ring (Frazer *et al.*, 1999). Hemispherical photographs were taken again during the third week of December 2005, because access to our study site was limited immediately following the hurricane.

Collection of fine roots

Fine roots were studied from a depth of 10 cm because soils at the study site are < 20 cm thick and are underlain by limestone bedrock (Vargas *et al.*, 2008). Three soil cores (10 cm depth and 4.5 cm in diameter) were collected at each exact location in which hemispherical pictures were taken in each one of the 60-m transects at all forest stands during the last week of September 2005. From each location, one core was used for radiocarbon analysis of live and dead fine roots, the second for radiocarbon analysis of live roots, and the third to measure fine root biomass (live and dead fine roots). Soil cores were collected again at the same locations during the third week of December 2005 (2 months after the hurricane) to assess fine root biomass of live and dead fine roots only.

Fine roots (< 2.0 mm in diameter) from all cores were sorted by hand and rinsed free of organic matter with deionized water and air dried afterwards. Subjective criteria (color, surface texture, brittleness) were used to separate live and dead roots. Live roots had an overall lighter color (white to brown), and dead roots were black. Live roots were less brittle and had a smooth surface texture.

During the last week of September 2005, approximately 3 wk before hurricane Wilma, we installed soil root in-growth cores to trap newly produced fine roots (van Noordwijk, 1993). The in-growth cores were buried (10 cm depth) in duplicates at each transect at two locations in which the hemispherical pictures and soil cores were taken. In-growth cores were made of PVC pipes (10 cm length) with a volume of 502.7 cm³, and the two openings (4 cm in diameter) were lined with a 1-mm

nylon mesh so that roots could grow into the cores. These cores were filled with soil collected *in situ* from each forest stand, sieved (2 mm mesh) and handpicked to remove roots. All in-growth cores were collected during the third week of December 2005 (2 months after the hurricane) and transported to the University of California, Riverside, CA, USA, where new live roots were handpicked as detailed above. No measurable dead roots were found in the in-growth cores. The use of soil in-growth cores often involves a lag of root growth into the root-free soil, and thus the new roots most likely grew during the 2 months after the hurricane when the trees were recovering from defoliation.

All collected roots (bulk, live and new) were from woody vegetation and no herbaceous plants were growing near the sampling points. In addition, all roots were from C_3 plants, as evidenced by their ^{13}C signatures (data not shown) and leaf anatomies. Dividing fine roots by plant species was logistically difficult because of the high species' richness at our study site (Table S1, see Supporting Information).

Radiocarbon analysis

Radiocarbon (^{14}C) was used to estimate the mean age of carbon in fine roots. Briefly, roots were treated with an acid–base–acid procedure to remove nonstructural carbon, oven dried at $65^\circ C$ and then ground (Gaudinski *et al.*, 2001). Samples were converted to graphite according to Xu *et al.* (2007) and measured for ^{14}C using accelerator mass spectrometry (AMS) at the UC Irvine W. M. Keck Carbon Cycle AMS facility (Trumbore *et al.*, 2006). To estimate the mean age of carbon in fine roots, we assumed that all structural carbon in the root grew in a single year and that the $\Delta^{14}C$ signature reflected the atmospheric $^{14}CO_2$ signature in that year. We did not separate the fine roots by branch order or soil depth, but previous studies have recognized the importance of these parameters to estimate root turnover (Joslin *et al.*, 2006; Guo *et al.*, 2008a).

The average age of the root carbon was determined by comparing the $\Delta^{14}C$ of the structural carbon of the roots with the record of $\Delta^{14}C$ of CO_2 in the atmosphere (Gaudinski *et al.*, 2001; Trumbore *et al.*, 2006). We used the $\Delta^{14}C$ record for tropical latitudes ($30^\circ N$ to $30^\circ S$) reported by Levin & Kromer (2004), and supplemented by measurements of remote marine air from these latitudes made between 2004 and 2006 (X. Xu & S. Tyler, pers. comm.). Calculated in this way, the mean age of structural tissues in roots will include any time lags associated with plant carbon storage (i.e. the age calculated is the time since the carbon used to grow the root was fixed from the atmosphere, and is the sum of plant storage and root lifetime for the mean population).

Fine root biomass

Live and dead fine roots were sorted by hand from soil cores and rinsed free of soil and organic matter to measure fine root biomass. The collected fine roots (live + dead) were oven dried

at $65^\circ C$ for 48 h to determine the total fine root biomass ($Mg\ ha^{-1}$) per forest stand before (September collection) and after (December collection) hurricane Wilma.

Statistical analyses

We used the mean of the three (hemispherical digital images, radiocarbon signature for live roots, live and dead roots, and fine root biomass) or four (radiocarbon signature of newly produced fine roots) measurements to represent the value of each transect (three transects per five forests). Datasets were tested for normality and arcsine transformed when needed. Paired t -tests were used to test for the hurricane effect (before and after) on canopy openness or fine root biomass in each one of the different stands (hypothesis 1). Simple linear regression was used to test whether the mean radiocarbon age of live, bulk or new roots increased with forest age (hypotheses 2, 3 and 4). ANCOVA was used to test for significant differences between slopes of radiocarbon age of bulk and live roots with forest age, or radiocarbon age of live and new fine roots with forest age (hypotheses 3 and 4). Data are presented as the mean \pm 1SD. All statistical analyses were performed using SPSS statistical software (SPSS Inc., v13.0, 2006, Chicago, IL, USA).

Results

Canopy openness before the hurricane varied substantially (between 5 and 11%) in the forest stands and, 2 months after the hurricane, canopy openness varied between 15 and 19% (Fig. 2). The immediate effect of sustained high winds on forest

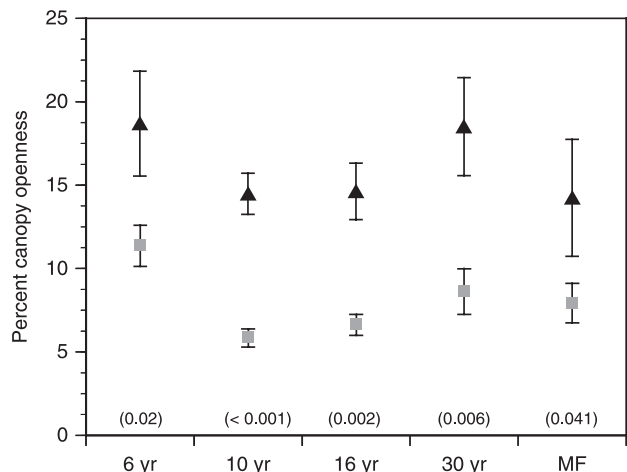


Fig. 2 Percentage canopy site openness 1 month before (squares) and 2 months after (triangles) hurricane Wilma in five seasonally dry tropical forests in the Yucatan Peninsula, Mexico. Values are means for tree transects at each forest ($\pm 1SD$), expressed as a percentage of canopy site openness calculated from hemispherical pictures and analyzed with the software Gap Light Analyzer. The numbers in parentheses represent P values of paired t -tests before and after the hurricane at each site. The x-axis represents the age of the forests from 6 yr to a mature forest (MF).

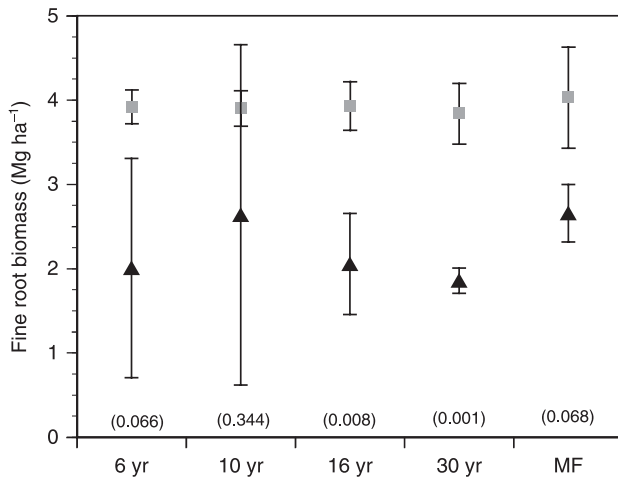


Fig. 3 Fine root biomass 1 month before (squares) and 2 months after (triangles) hurricane Wilma in five seasonally dry tropical forests in the Yucatan Peninsula, Mexico. Values are means for three transects at each forest (± 1 SD). The numbers in parentheses represent P values of paired t -tests before and after the hurricane at each site. The x-axis represents the age of the forests from 6 yr to a mature forest (MF).

vegetation was the complete denudation of leaf biomass and the uprooting of some trees (Fig. S1, see Supporting Information). As a result, we found significant ($P < 0.05$) increases in canopy openness in all forest stands (Fig. 2).

Fine root biomass before the hurricane had an overall mean of 3.9 Mg ha^{-1} among forest stands. Two months after the hurricane, the overall mean of fine root biomass was significantly ($P < 0.001$) reduced (by 43%) to 2.3 Mg ha^{-1} among forest stands. For individual forest stands, fine root biomass decreased significantly ($P < 0.01$) in the 16- and 30-yr-old forests and decreased marginally ($P < 0.1$) in the 6-yr-old and mature forests (Fig. 3).

Radiocarbon values ($\Delta^{14}\text{C}$) of bulk fine roots (live + dead roots) increased significantly with forest age ($P < 0.001$, $r^2 = 0.91$) and above-ground biomass ($P < 0.001$, $r^2 = 0.896$). The value of $\Delta^{14}\text{C}$ of bulk fine roots in young forests (6 and 10 yr) was nearly 85‰ (mean age of 4 yr), but increased to 120‰ (mean age of 11 yr) in mature forest (Fig. 4a).

Live fine roots collected before hurricane Wilma also increased in $\Delta^{14}\text{C}$ values with forest age ($P = 0.002$, $r^2 = 0.55$) and above-ground biomass ($P = 0.001$, $r^2 = 0.577$). Live fine roots of young forests (6 and 10 yr) had $\Delta^{14}\text{C}$ values of nearly 65‰ (Fig. 4a,b). The mean $\Delta^{14}\text{C}$ of atmospheric CO_2 for tropical latitudes (30°N to 30°S) during 2005 was $67 \pm 2\text{‰}$ (X. Xu & S. Tyler, pers. comm.), suggesting that these live roots were produced with recently fixed carbon (< 1 yr old). Fine roots of the forests older than 16 yr had mean $\Delta^{14}\text{C}$ values that ranged from 73 to 77‰ , suggesting that these live roots were also produced with recently fixed carbon (< 2 yr old; Fig. 4a,b).

In all forest stands, $\Delta^{14}\text{C}$ values of live fine roots were substantially lower than the $\Delta^{14}\text{C}$ values of bulk fine roots. Using

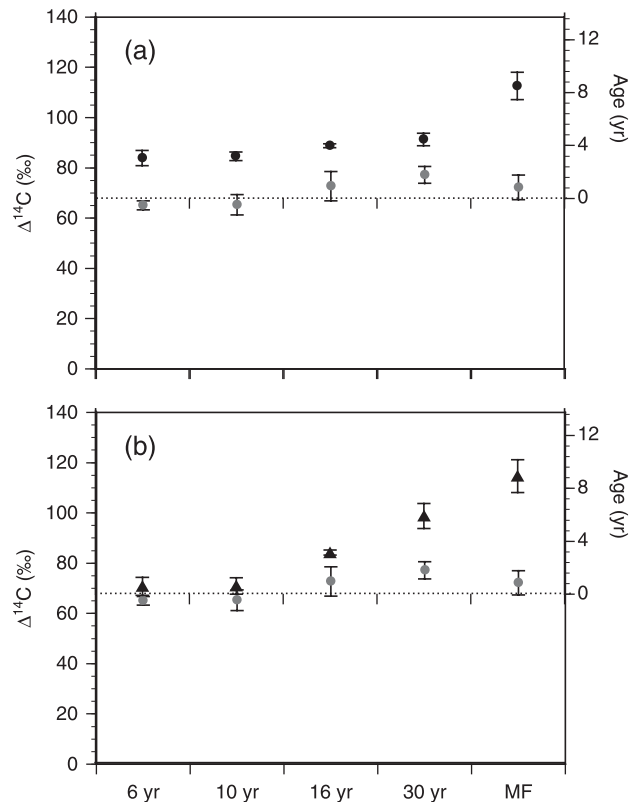


Fig. 4 Radiocarbon values and the estimated age of carbon in fine root structural tissues of fine roots (< 2 mm) sampled before and after hurricane Wilma using $\Delta^{14}\text{C}$ of CO_2 . Values are means for three transects at each forest (± 1 SD) for bulk (live + dead) roots (black circles) and live roots (grey circles) sampled 20 d before the hurricane (a), and live roots (grey circles) sampled 20 d before the hurricane and new fine roots (triangles) produced up to 2 months after the hurricane (b). The broken line represents atmospheric $\Delta^{14}\text{C}$ for 2005. The x-axis represents the age of the forests from 6 yr to a mature forest (MF). All samples of fine roots (bulk, live and new) were from C_3 plants, as revealed by their ^{13}C signatures (data not shown).

ANCOVA, we found significant differences between the slopes of $\Delta^{14}\text{C}$ values of live roots and bulk roots with forest age ($F_{2,30} = 95.08$, $P < 0.001$) and above-ground biomass ($F_{2,30} = 96.665$, $P < 0.001$), showing significantly lower $\Delta^{14}\text{C}$ values in live fine roots (Fig. 4a).

New live fine roots, recovered from in-growth cores after the hurricane, showed increased $\Delta^{14}\text{C}$ values with forest age ($P < 0.001$, $r^2 = 0.902$) and above-ground biomass ($P < 0.001$, $r^2 = 0.899$). The value of $\Delta^{14}\text{C}$ in young forests (6 and 10 yr) was close to 70‰ , which corresponds to an expected mean age of newly produced fine roots of nearly 2 yr. By contrast, new live fine root carbon in the 16-yr-old forest increased to nearly 85‰ , or a mean age of 4 yr. It was noteworthy that new live fine roots of the 30-yr-old and mature forests had mean $\Delta^{14}\text{C}$ values of nearly 95 and 115‰ , or mean ages of nearly 7 and 10 yr, respectively (Fig. 4b).

In all forest stands, $\Delta^{14}\text{C}$ values of new fine roots (produced after the hurricane) were substantially higher than $\Delta^{14}\text{C}$ values of live fine roots sampled before the hurricane. Using ANCOVA, we found significant differences between the slopes of $\Delta^{14}\text{C}$ values of new fine roots and live fine roots with forest age ($F_{2,30} = 41.077, P < 0.001$) and above-ground biomass ($F_{2,30} = 42.187, P < 0.001$), showing significantly higher $\Delta^{14}\text{C}$ values in new fine roots produced after the hurricane (Fig. 4b).

Discussion

The possibility that plants allocate older stored carbon for the production of new fine roots provides a new dimension of the fate and the implied role of stored carbon in plants. In this study, we had the unusual opportunity of sampling fine roots and installing root in-growth cores 3 wk before the unexpected passage of hurricane Wilma. Because the use of soil in-growth cores often involves a lag of root growth into the root-free soil, the new fine roots most likely grew during the 2 months after the hurricane when the trees were recovering from defoliation. Our results provide evidence that plants have the capacity to re-mobilize older reserves of carbon for the production of fine roots following a severe wind disturbance. Furthermore, the capacity to allocate older stored carbon for the production of new roots increased with forest age and forest above-ground biomass stocks. These results have further implications on how plants use stored carbon, root turnover dynamics and plant–ecosystem resilience.

Our results support hypothesis (1) because the immediate effect of the hurricane at our study site was the denudation of vegetation and decrease in fine root biomass. Our results are comparable with those of previous studies with a decrease in the leaf area index and fine root biomass following hurricanes (Silver & Vogt, 1993; Beard *et al.*, 2005). The loss of leaves and fine roots limits the capacity to photosynthesize and to take up nutrients and water, which may further reduce ecosystem gross primary productivity following a hurricane disturbance (Li *et al.*, 2007).

Our results support hypothesis (2) as the mean radiocarbon age of bulk fine roots was between 4 and 11 yr and increased with forest age and above-ground biomass. The radiocarbon age of bulk fine roots is consistent with observations from previous studies in temperate (Gaudinski *et al.*, 2001; Hogberg *et al.*, 2002; Tierney & Fahey, 2002; Guo *et al.*, 2004; Joslin *et al.*, 2006; Keel *et al.*, 2006; Vargas & Allen, 2008b) and tropical (Trumbore *et al.*, 2006; Vargas *et al.*, 2009) forests.

Our results support hypothesis (3) because the radiocarbon values of live roots before the hurricane showed that they were produced from recent photosynthetic products (between 1 and 2 yr), and are consistent with previous studies (Gaudinski *et al.*, 2001; Matamala *et al.*, 2003; Joslin *et al.*, 2006; Keel *et al.*, 2006; Trumbore *et al.*, 2006). It is noteworthy that our values were lower than the mean ages of live roots found in other tropical forests (Trumbore *et al.*, 2006). However, these authors

identified differences in root sorting methodologies as a potential factor in the radiocarbon results. Our results suggest that, although live roots were produced from recent photosynthetic products, radiocarbon values increased with forest age and above-ground biomass. These results suggest that: a) older and larger plants may produce new roots from recent photosynthetic products but root turnover decrease with forest age, or b) these plants have the capacity to use a combination of recent and relatively older carbon (< 3 yr) for the production of new fine roots.

Our results do not support hypothesis (4) because radiocarbon values were higher than the expected observations for new fine roots being constructed from recent photosynthetic products of < 2 yr of age (Gaudinski *et al.*, 2001; Matamala *et al.*, 2003; Joslin *et al.*, 2006; Keel *et al.*, 2006; Trumbore *et al.*, 2006). Although we did not measure the carbon reserves (e.g. nonstructural carbohydrates) directly in plants, our results suggest that the most likely explanation for these observations is that plants have the capacity to re-allocate older stored carbon for the production of new fine roots following leaf defoliation and fine root mortality.

Previous studies have provided evidence that older or stored photosynthetic products may be used for root production and root metabolism. First, it has been reported that, in mature black spruce (*Picea mariana*)-dominated boreal forests, root respired CO_2 shows $\Delta^{14}\text{C}$ values significantly higher than those of recent photosynthetic products (Czimczik *et al.*, 2006; Schuur & Trumbore, 2006), suggesting that NSC pools of several years of age are used in root metabolism and/or growth. Second, it has been observed that environmental stress conditions influence the size of NSC pools (Wurth *et al.*, 2005) that can be used for the production of fine roots (Guo *et al.*, 2004). Third, it has been observed that re-mobilization from below-ground carbon stores may support the production of fine roots in systems with severe and frequent disturbances (Langley *et al.*, 2002). Thus, all of these studies support our interpretation that, under stressful conditions, plants may have the ability to re-allocate carbon reserves for the production of new fine roots. This capacity could be an adaptation of plants in SDTFs as they have evolved under conditions of annual dry seasons, as well as recurrent large-scale disturbances, such as fires or hurricanes.

Our results provide evidence that newly produced roots are constructed from increasingly older carbon during forest ecosystem development. We propose that, at young sites (6- and 10-yr of age), new roots are produced with recently fixed stored carbon (~2 yr of age), whereas, in older forests, new roots are produced with older stored carbon (up to 10 yr of age). These results suggest that the age, size and volume of the plant (e.g. higher basal area and tree height in older forests; Table 1) may influence the capacity to re-allocate stored carbon because of the potential magnitude of NSC associated with tissue volume (Körner, 2003; Wurth *et al.*, 2005; Poorter & Kitajima, 2007).

We propose that species' changes across the chronosequence following disturbance are less likely to be a cause of differences in ages of newly produced fine roots. Because of the high plant diversity at our study site, separation by plant species was logistically difficult, although admittedly different plant species probably have differential capacities to allocate old stored carbon. However, there are no dominant plant species at the study site (Schultz, 2005), and we did not see substantial changes in species' richness following the hurricane (Table S1, see Supporting Information).

Our study could be improved by installing subsequent in-growth cores with time since the hurricane. This approach could determine when trees return to use new carbon to produce new fine roots following the recovery of the canopy. It would be useful to account for fine root branch order and dividing roots by plant species as suggested in previous studies (Guo *et al.*, 2008a,b). Finally, it is critical to directly assess the carbon reserves themselves to provide direct evidence of the proposed mechanism that re-mobilization of older carbon reserves occurs.

Our results have further implications for fine root turnover dynamics. We found that bulk fine roots have mean ages of 4–11 yr, but live roots have mean ages of 1–2 yr. These results suggest the possibility that, in our study site, root turnover may be relatively high and root decomposition slow, probably as a result of seasonality in rainfall. This explanation is supported by the radiocarbon signatures of live roots, but assumes that all new roots were produced with recently fixed carbon, and therefore the higher $\Delta^{14}\text{C}$ values reflect the importance of dead undecomposed roots. An alternative explanation is that bulk fine roots may contain a high proportion of fine roots that were constructed with older stored carbon (during past stressful events), which would inflate the $\Delta^{14}\text{C}$ values.

It is critical to understand how plants allocate older carbon for the production of fine roots, and under which circumstances plants possess this adaptation. Furthermore, it is important to determine how large is the proportion of older carbon allocated for the production of new roots, and how to consider these pools for root dynamics (Luo, 2003; Matamala *et al.*, 2003, 2004; Luo *et al.*, 2004). These goals have been considered in previous studies by recognizing the importance of fine root heterogeneity in branch order (Gaudinski *et al.*, 2001; Guo *et al.*, 2008a), and the possibility of a bimodal age distribution of live roots (long-lived roots and roots with short turnover times) (Tierney & Fahey, 2002; Joslin *et al.*, 2006). In addition, Luo (2003) has recognized the possibility that carbon could spend a significant amount of time in storage pools, and our results show that this stored carbon can be used following a disturbance.

The adaptation to use stored carbon has important implications for fine root production or the generation of other recalcitrant structures following disturbances (Landhausser & Lieffers, 2002; Guo *et al.*, 2008b), but, to our knowledge, the age of these carbon pools has not been quantified. Defoliation and root mortality represent a carbon cost to the plant that may reduce gross primary productivity (Li *et al.*, 2007) and photo-

synthate use to support roots and mycorrhizae. Thus, plants that have the capacity to allocate stored carbon as a resource to rapidly produce fine roots and maintain mycorrhizal fungi following stress conditions and disturbances would increase their chance for recovery and survival.

Finally, it is crucial to understand the relationship between disturbance intensity and frequency, as multiple disturbances may deplete stored carbon reserves (Landhausser & Lieffers, 2002) and could determine an ecosystem's ability to recover (Beard *et al.*, 2005). The intensity, type and frequency of the disturbance may influence the relationships between carbon assimilation and root carbon utilization (Hogberg *et al.*, 2002; Guo *et al.*, 2004; Keel *et al.*, 2006). Clearly, further research is needed to improve the understanding of the importance of stored carbon for plant–ecosystem carbon dynamics.

Conclusions

This study is one of the few to have measured fine root radiocarbon ages in tropical forests. We report ages of between 4 and 11 yr for bulk roots (mixed live and dead fine roots) in seasonally dry tropical forests. Pre-hurricane live fine roots were younger (1–2 yr) than bulk roots and appeared to be made with mostly recently fixed carbon. The novelty of this study is that, contrary to expectations, new live fine root radiocarbon ages increased after a severe hurricane disturbance (new root ages between 2 and 10 yr), which is only possible if these new live roots grew from old carbon reserves of plants. Our results provide a new dimension of the fate of stored carbon in plants, and have implications for root turnover dynamics and plant–ecosystem resilience.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Image of a 16-yr-old seasonally dry tropical forest 3 wk after the pass of hurricane Wilma.

Table S1 Species' richness before and after hurricane Wilma in five different forest stands of a seasonally dry tropical forest in the Yucatan Peninsula, Mexico

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