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Stable carbon isotope signatures of ancient Maize agriculture at El Kinel, Guatemala

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Stable C isotope studies of the soil organic matter (SOM) have delineated areas with histories of vegetation change from C₃ forest to C₄ maize (*Zea mays* L.) agriculture and back to the contemporary C₃ forest. The objectives of this study were to: (1) determine if land around El Kinel, Guatemala possessed a vegetative history of shifts from C₃ forest to C₄ maize agriculture in the past, (2) determine if 10 years of contemporary maize production is sufficient time to deposit an isotopic signature of C₄ plants in the root zone (top 40 cm), and (3) to examine the extractable phosphorus concentrations and $\delta^{13}\text{C}$ in soils of important archaeological features that included a midden, a burial, and two ancient reservoirs (aguadas). The lack of a shift in $\delta^{13}\text{C}$ greater than 3.5‰ in the top 40 cm of the contemporary maize field suggested that continual maize cultivation of more than ten years is required to create an isotopic signature for maize agriculture. Carbon isotopic evidence was found in soil profiles to confirm that long-term agriculture was practiced by ancient Maya farmers at El Kinel. The man-made aguadas did not show isotopic shifts greater than 2.3‰ in any part of the profile, indicating they were used for other purposes not associated with C₄ plant growth. The relatively low P (<30 mg kg⁻¹) was found in soil at the same depth but at a distance of 30 cm from an ancient burial. The high P concentration (127 mg kg⁻¹) found within millimeters of the bones implied that the P enrichment came from the remains but P remained fixed in the soil and did not migrate.

Key words: C₃ and C₄ plants, humin, rhizodeposition, Maya archaeology, phosphorus

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Introduction

Archaeology like many other disciplines is ever evolving and becoming more of a multidisciplinary field. Soil geochemistry is one tool that has been used to discover anthropogenic residues of ancient activities (Entwistle et al. 2000; Terry et al. 2004; Holliday and Gartner 2007). The focus of this paper is the use of stable carbon isotopes of the soil organic matter (SOM) to find the signatures of ancient vegetation shifts from native forest growth to forest clearance for maize (*Zea mays* L.) agriculture. A brief description of the effects of shifts from C₃ forest vegetation to C₄ plants associated with maize agriculture are provided along with sample collection and preparation methods. Soil pedons from recently cleared maize fields were used in a case study of isotopic signatures of contemporary and ancient maize agriculture on the border of Guatemala and Mexico at El Kinel (Fig. 1).

In the absence of agricultural structures such as terraces it is difficult to discover land use strategies and agricultural methods that sustained the ancient Maya. Stable carbon isotope analyses of skeletal remains have provided evidence that

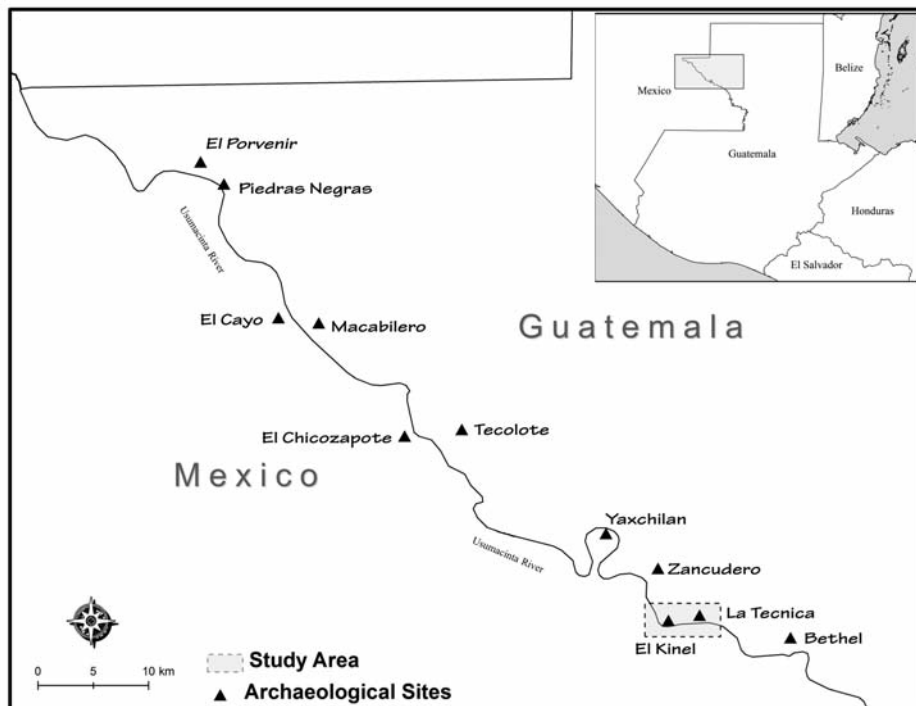


Fig. 1
Map of the Usumacinta River basin on the border of Guatemala and Mexico. The locations of the sites of El Kinel and La Técnica are shown

maize, a C₄ plant, was an important food source among the ancient Maya populations (Wright and White 1996; Tykot 2002; Scherer et al. 2007; López et al. 2011). However, skeletal remains do not inform us how or where the food was produced. One of the reasons that it is difficult to understand where agriculture took place is the lack of prehispanic records (Wilken 1971). Additionally, agricultural activities seldom left architectural or artifactual evidence to be discovered (Burnett et al. 2012a; Burnett et al. 2012b). Further complicating our understanding of food production is the immense area and the diverse landscapes occupied by the ancient Maya. The paucity of archaeological evidence for ancient agricultural activities has led to multiple hypotheses on the subject of Maya food production ranging from widespread deforestation for swidden culture of maize, to maize as a supplement to ramon nut and other crops (Folan et al. 1979; Puleston 1982).

To gain a better understanding of where ancient agriculture took place in Mesoamerica and the Caribbean basin, stable C isotope studies of the SOM have been applied to delineate areas with histories of vegetation change from C₃ forest to C₄ maize agriculture and back to the contemporary C₃ forest (Lane et al. 2004; Webb et al. 2004; Fernandez et al. 2005; Johnson et al. 2007; Webb et al. 2007; Beach et al. 2008; Wright et al. 2009; Burnett et al. 2012a b).

Stable carbon isotopes and root zone SOM

The idea behind using stable C isotopes in SOM for identification of ancient maize agriculture is based in the differing isotopic discrimination against heavy carbon (¹³C) between plants commonly associated with forests versus many of the tropical grasses (Farquhar et al. 1989b). Plants that utilize the Hatch-Slack pathway (C₄, grasses) for carbon assimilation discriminate less against ¹³CO₂ than those that use the Calvin Cycle (C₃, woody plants) (Smith and Epstein 1971; Farquhar et al. 1982; Cerling et al. 1997). Atmospheric CO₂ contains 1.1 atom percent ¹³C (Farquhar et al. 1982; Farquhar et al. 1989a). Plant membranes and photosynthetic pathways isotopically discriminate against the heavier ¹³CO₂ (compared to that of ¹²CO₂), making their δ¹³C lower than that of the atmosphere (Farquhar et al. 1982). Plant isotopic discrimination against ¹³C is defined as the ratio of ¹³C to ¹²C compared to that of the standard, Pee Dee Belemnite (PDB) (Cerling et al. 1997):

$$\left(\% \delta^{13}\text{C} = \left(\frac{\frac{^{13}\text{C}}{^{12}\text{C}} \text{ Sample}}{\left(\frac{^{13}\text{C}}{^{12}\text{C}} \text{ PDB} - 1 \right) \times 1,000} \right) \right)$$

Under the current atmospheric CO₂ mixing-ratio and ¹³CO₂ conditions, C₃ plants have an overall δ¹³C of -30 to -20‰ with an average of -27‰ compared

to that of the C_4 plants with $\delta^{13}C$ values of -14 to -10‰ averaging -12‰ (Bender 1968; Cerling et al. 1997; Liu et al. 1997).

Deposition of ^{13}C from the plant to the SOM is accomplished by decomposition of above-ground tissues (Balesdent et al. 1988), below-ground biomass, and rhizodeposition (Kuzyakov and Domanski 2000; Amos and Walters 2006) (Fig. 2). The overall contribution of C to the SOM from above-ground tissues, below-ground biomass and rhizodeposition varies. After a 15-year study, Bolinder et al. (1999) gave estimates for C incorporated from maize above-ground and below-ground biomass to the SOM at 77 to 20% and 16 to 30%, respectively. This and other studies have shown that a greater portion of maize carbon retained in the SOM originated from roots than from shoots (Balesdent and Balabane 1996; Molina et al. 2001). Rhizodeposition continually contributes C into the SOM accounting for 5-21% of all photosynthetically fixed carbon released into the soil (Marschner 1995). Furthermore, in an anthropogenic setting where crops are removed from the field the above-ground biomass contribution could be even lower than the above listed values.

Once the plant carbon is incorporated into the pool of SOM, the isotopic signature is preserved, though microbial digenesis may cause an isotopic

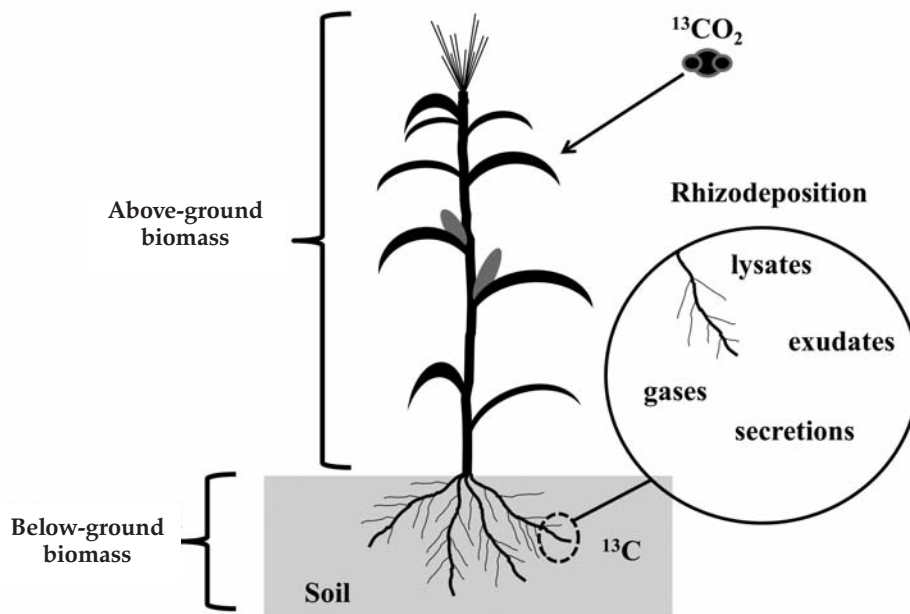


Fig. 2
Deposition of ^{13}C from a maize plant to the SOM is accomplished by decomposition of above-ground tissues, below-ground biomass, and rhizodeposition

enrichment in ^{13}C of about 2.5‰ (Balesdent and Mariotti 1987; Agren et al. 1996; Boutton 1996; Cerling et al. 1997). The amount of time it takes to "create" an isotopic signature of vegetation type in the soil is not fully understood. However, soil composition and environmental conditions influence the amount of time needed for an isotopic signature to change. For example, Vitorello et al. (1989) found that twelve years after a forest (C_3 plants) was converted to C_4 sugarcane (*saccharum*), 90% of the carbon in the clay fraction still had the forest signature, demonstrating the ability of the clay to preserve the isotopic signature. A more in depth review of the process of ^{13}C deposition by maize can be found in Balzotti et al. (2013).

Case study of agriculture soils at El Kinel

The Usumacinta valley between Guatemala and Mexico has provided valuable soil resources for the ancient Maya as well as for contemporary Maya farmers. The flood plain soils reach depths of over 3 m and are relatively free of rock. Soils of the valley have been examined in profile at archaeological test pits and in addition soil pedons have been examined by bucket auger collection. A pedon is defined as an individual soil, whereas a soil profile in an archaeological pit can be viewed as undisturbed soil horizons. Soil properties and the $\delta^{13}\text{C}$ of horizons from 39 soil pedons near the sites of El Porvenir, Piedras Negras and the Rio Macabilero drainage have been reported (Fernandez et al. 2005; Johnson et al. 2007). These sites are on the Guatemalan side of the Usumacinta River and extend north from El Kinel to within 20 km of the Mexican border to the north (Fig. 1). These studies have identified ancient maize agricultural activity on the Usumacinta River Basin. The $\delta^{13}\text{C}$ results suggested that there are strong signatures of C_4 plants, such as maize and tropical grasses in many of the soils, especially in the foot and toeslopes throughout the study area. Further, the current soil conditions and extent of relatively gently sloped areas are favorable for agriculture. Overall, the results are supportive of the hypothesis that the area, located between the polities of Piedras Negras and Yaxchilán, was agriculturally important to the surrounding region.

The archaeological site El Kinel, found near the contemporary village of La Técnica, Guatemala (Fig. 3), is located in a modern maize field where local farmers have cultivated maize for 10 continuous years without the addition of fertilizers or pesticides (Golden and Scherer 2006; Houston et al. 2006; Golden et al. 2008). Neighbouring farms outside the river valley with shallow karst soils are fortunate if they produce for 3 continuous years without a fallow period. This unique ability of flood plain soils to sustain maize cultivation provided an opportunity to determine whether 10 years of continual maize production is sufficient to shift the isotopic signature more than 3.5‰ in this area.

Local farmers from the nearby community of La Técnica cleared the site of El Kinel for agricultural use starting in the 1990s. The deep alluvial soils at the site

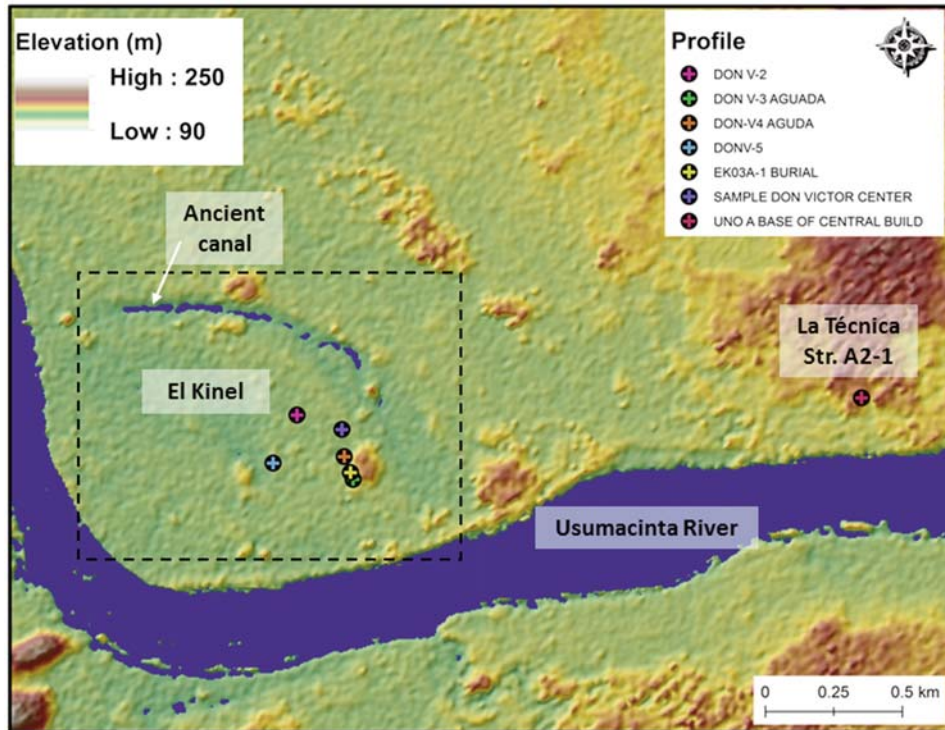


Fig. 3
Digital elevation model of the sites of El Kinel and La Técnica, Guatemala on the banks of the Usumacinta River. The locations of soil pedons and the ancient canal are shown

had sustained maize agriculture for 10 years prior to sampling in 2006 (Don Victor Ramirez Carreto, personal communication). The ancient canal at the site (Fig. 4) could have functioned as both a water-management feature and a defensive feature (Houston et al. 2006). The canal separates land at a bend in the river from the mainland and anciently would have created an island before the canal was largely filled by sediment.

Rooting depths for maize vary and can reach beyond 1 m. However, in a review by Amos and Walters (2006) it was reported that between 70–90% of the root biomass can be found in the top of 0.3 m of the soil (Aina and Fapohunda 1986; Crozier and King 1993; Dwyer et al. 1996). At the site of El Kinel archaeologists only encountered cultural material below depths of 30 to 40 cm in test pits. It is likely that much of the site was buried in nearly 40 cm of flood sediment during the past 1100 years of abandonment. Therefore, a shift in $\delta^{13}\text{C}$ from the surface to 0.4 m in this study can be attributed to modern maize ^{13}C input.

The objective of this study was to: (i) determine if the areas in and around El Kinel showed a shift from C₃ to C₄ plants in the past, (ii) determine if 10 years of contemporary maize production is sufficient time to deposit a C₄ signature in the root zone (top 40 cm), and (iii) examine the extractable phosphorus concentrations and ¹³C of important archaeological features such as a waste midden, a burial (subsequently covered in midden material), and two small water catchment reservoirs (aguadas).

Materials and Methods

Soil pedon collection

Five soil pedons at El Kinel were collected by bucket auger and examined. Three sampling locations within the contemporary maize field were thought to have high agricultural potential based on low slope, deep soils, short distance to water, and proximity to ancient structures (Don V-center, Don V-2 and Don V-5). Two pedons thought to have low agricultural potential were collected in the silted remains of two small reservoir basins, called aguadas, near the highest concentration of structures (Don V-3 and Don V-4). The aguadas are depression areas that currently contain wetland vegetation. Agricultural and aguada soil samples were collected with an 8-cm diameter bucket auger at 15-cm depth increments, beginning with the surface. For each agriculture and aguada pedons a depth of one to three-m was reached providing between 10 and 20 samples per pedon with an overall total of 86 horizon samples. The soil horizon characteristics of two profiles were examined within archaeological test pits. Horizon samples were collected at 10-cm depth intervals from open archeological pits near structures: a midden at the base of Structure A2-1 at La Técnica (Quiroa et al. 2006) and a midden that contained a burial at El Kinel (Pérez Robles and Arroyave 2007: 42–43; Burial 3; Fig. 3). Soil samples contained in polyethylene bags were transported to the Brigham Young University Environmental Analysis Laboratory (Provo, UT). The slope and elevation of the profile and pedon locations were measured on site, using a clinometer and on computer by a digital elevation model (DEM) generated from data collected by Airborne Synthetic Aperture Radar (AIRSAR) (Evans et al. 1986) and Shuttle Radar Topography Mission (SRTM) (Farr et al. 2007). The same DEM data were used to determine the landscape position of each soil. The Universal Transverse Mercator (UTM) coordinates of each pedon and profile were recorded in the field.

Laboratory Analysis

Samples were air-dried and crushed to pass a 2 mm (10 mesh) sieve. Soil texture was determined using the hydrometer method of Gee and Bauder (1986). The pH of each surface horizon (~ 0–15 cm) was determined on a 1:2 soil to water mixture by glass electrode. The Mehlich extractable phosphorus (P) levels were also

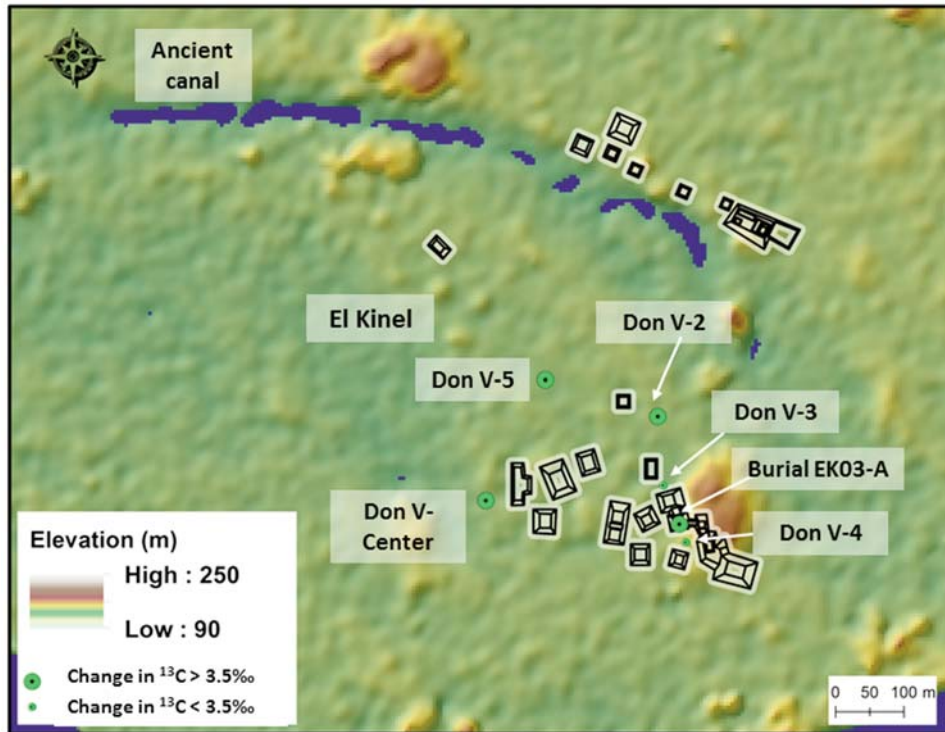


Fig. 4
Digital elevation model and map of the site of El Kinel, Guatemala. The locations of structures, soil pedons and the ancient canal are shown

measured for those same horizons (Terry et al. 2000). Five-gram sub-samples were further ground to pass a 250 μm (60 mesh) sieve in preparation for stable C isotope analyses.

Carbonates were removed from the 60-mesh samples by reaction with excess HCl and rinsing, prior to C isotope analysis. Researchers have raised concern that some pre-analysis acidification procedures for removal of carbonates cause significant, non-systematic bias in the isotope analysis (Brodie et al. 2011). To address these concerns, samples were centrifuged at high speed ($>30,000 \times g$ for more than 1 hour) after acidification and after rinsing with water. Based on the findings of Webb et al. (2004) that the humin fraction would be the most sensitive detector of ancient C_4 vegetation in this type of environment, the humic acid and fulvic acid fractions were removed by alkaline pyrophosphate extraction following Webb et al. (2004; 2007) and Wright et al. (2009). The stable C isotope ratios of the humin fraction of the SOM of each soil horizon were determined by isotope-ratio mass spectrometer (Thermo Finnigan Delta V, Waltham, MA) coupled with an elemental analyzer (EAIRMS) (Costech, Valencia, CA). The

standard deviation of six replicate analyses of $\delta^{13}\text{C}$ was 0.27‰. The stable C isotope values of the humin fractions were reported as $\delta^{13}\text{C}$ in per mil notation (‰).

The absolute value of the largest shift in $\delta^{13}\text{C}$ values between surface and subsurface horizons was reported for each pedon as the change in $\delta^{13}\text{C}$. This value represents the amount of ^{13}C enrichment in each soil and was the primary indicator used to measure the effects of past vegetation changes on the SOM.

Results

Surface samples from the contemporary maize field pedons (Don V-Center, Don V-2 and Don V-5) were enriched in ^{13}C by an average of 1.5‰ and none of the three pedons exhibited $\delta^{13}\text{C}$ shifts greater than 3.5‰ in the top 40-cm zone of maximum root activity (Fig. 5). However, all three possessed shifts greater than 3.5‰ in the lower depths that were likely associated with changes in vegetative history. The greatest shift was found in pedon Don V-5 (5.1‰) at the 67-cm depth (Fig. 5). A second shift in $\delta^{13}\text{C}$ was 4.0‰ was observed at the 202-cm depth. Shifts of more than 3.5‰ were found at depths of 115 and 283 cm in pedon Don V-

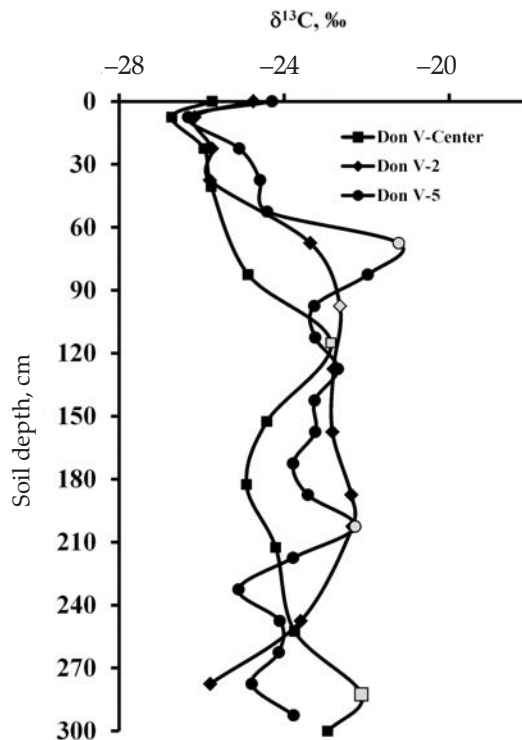


Fig. 5
The change in $\delta^{13}\text{C}$ with soil depth of the humin fraction of pedons Don V-Center, Don V-2 and Don V-5. These pedons were located in a field cropped to maize for 10 years

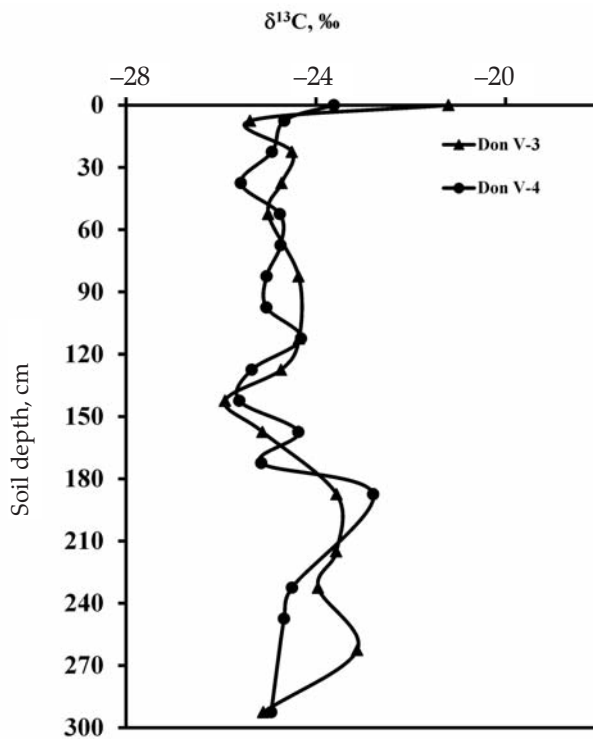


Fig. 6
The change in $\delta^{13}\text{C}$ with soil depth of the humin fraction of pedons Don V-3 and Don V-4. These pedons were located in two ancient water reservoirs adjacent to house mounds at the site of El Kinel

Center and at depths of 97 and 202 cm in pedon Don V-2 (Fig. 5). These shifts in $\delta^{13}\text{C}$ below the contemporary zone of maximum rooting activity suggest ancient shifts in vegetation from forest to maize agriculture at two periods in antiquity.

Neither of the pedons from the aguadas (Don V-3 and Don V-4) exhibited shifts in $\delta^{13}\text{C}$ greater than 3.5‰ at any depth (Fig. 6). The greatest shift in $\delta^{13}\text{C}$ was 2.3‰ in pedon Don V-3 at a depth of 262 cm. The soil profiles exposed at two test pits located in middens adjacent to structures at El Kinel (Burial 3) and nearby La Técnica (Str. A2-1) were examined for changes in extractable P and ^{13}C enrichment with depth. The structures and test pits were higher in elevation (Fig. 2) and were not affected as much by flood deposition. Midden material was found to a depth of 110 cm at the base of structure A2-1 at La Técnica. The extractable P concentrations in the profile exceeded 120 mg kg^{-1} at the 40 to 80-cm depth (Fig. 7). Soil in this midden possessed the signature of C_4 vegetation in all but the surface 10 cm. A burial was found at the bottom of the test pit at El Kinel (EK-03 A) (Pérez Robles and Arroyave 2006). The midden material above the burial at El Kinel was enriched in P ranging from 23 to 30 mg kg^{-1} at the 40 to 60-cm depths. Soil at the same depth as the burial but 30 cm away from the bones was low in P, ranging from 10 to 16 mg kg^{-1} . However, a separate soil sample taken adjacent to the shoulder bones of the burial contained 127 mg kg^{-1} P.

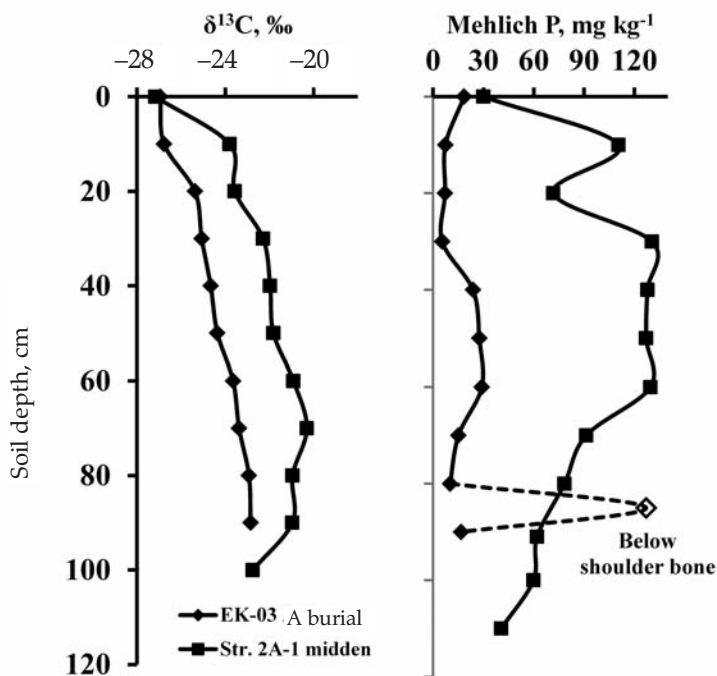


Fig. 7 The changes in Mehlich extractable P concentration (mg kg^{-1}) and in $\delta^{13}\text{C}$ (‰) with profile depth of midden test pits at La Técnica Structure A2-1 and at burial EK-03 A

Discussion

None of the modern maize field pedons showed shifts greater than 3.5‰ in the zone of maximum root activity for maize (top 40 cm). This lack of shifts suggest that continual maize cultivation of greater than 10 years would be required to deposit sufficient ^{13}C into the SOM.

Enrichment in ^{13}C greater than 3.5‰ at the 67 to 115-cm depths and again at the 2 to 3-m depths of the contemporary maize field pedons suggested ancient shifts in vegetation from forest to maize agriculture at two periods in antiquity. The two C_4 vegetation shifts with intervening C_3 forest vegetation provided evidence of maize cultivation during the Preclassic period and again in the Classic Maya period, about 3000 to 1700 years before present (BP) and about 1400 to 1100 years BP, respectively.

Archaeologists excavated a 1 m × 1 m × 3-m deep test pit at the location of the aguada pedon Don V-3 (Pérez Robles and Arroyave 2006: 47). No artifacts were

found in the test pit until the 3-m depth was reached. Beach sands were found at that depth with several large potsherds. It can reasonably be assumed that the normally flooded aguada soils have never been used in maize agriculture and serve as a comparison for the background change in $\delta^{13}\text{C}$ attributed to flood deposited sediments. The lack of $\delta^{13}\text{C}$ shifts greater than 3.5‰ within the aguadas further validates the lack of maize agriculture in these features.

The soil P accumulation and ^{13}C enrichment in the two midden test pits suggested that significant portions of the waste materials in the middens were high in both P and in ^{13}C from C_4 plant-derived foodstuffs. The low P concentration at the depth of the burial but 30 cm from the bones compared to soils collected within millimeters of the bones illustrated that P became fixed to soil particles and did not move more than a few cm from its source in the soil. The change in $\delta^{13}\text{C}$ of more than 3.5‰ was observed at the 70- to 90-cm depths, closest to the burial. The midden materials, residues from the burial and organic matter from ancient maize agriculture were sources of the ^{13}C enrichment but the proportional contributions of each source are not known. No modern fertilizers had been used in or around the site suggesting that the P enrichment of the midden and burial profiles was from the deposition of food and other waste materials. The relatively low P content ($<30 \text{ mg kg}^{-1}$) of soil above and below the burial, the high P concentrations (127 mg kg^{-1}) found within millimeters of the bones implies that the P enrichment came from the remains. Phosphorus remained fixed in the soil within the burial and did not migrate.

Conclusions

Combined with the proximity to residential structures, deep soils, and high agricultural potential, the pedons Don V-Center, Don V-2 and Don V-5 yielded carbon isotopic evidence that long-term agriculture was practiced by the ancient inhabitants of El Kinel. Furthermore, the man-made aguadas did not show isotopic shifts of vegetation change, indicating they were used for purposes other than maize cultivation. The lack of a shift in $\delta^{13}\text{C}$ greater than 3.5‰ in the top 40 cm of the contemporary maize field suggested that continual maize cultivation of more than ten years is required in this region to create an isotopic signature for maize agriculture in the SOM. These data confirm that the soils of the Usumacinta valley were important agriculture resources that sustained the ancient Maya.

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Table 1
Properties of soil pedons from the sites of El Kinel and La Técnica, Guatemala on the Usumacinta River. Physical and chemical properties of surface horizons are shown. The maximum changes in $\delta^{13}\text{C}$ with pedon depth are listed

Soil Pedon	Pedon	Soil Color	Surface 0–15 cm					Change				
			Sand	Silt	Clay	Texture	Class†	pH	Mehlich P	$\delta^{13}\text{C}$ %	$\delta^{13}\text{C}$ in	UTM Zone 12S
Don V-Center	300	10YR5/3	446	318	236	L	6.2	11.7	-25.0	-2.1	722341	1861610
Don V-2	278	10YR5/3	469	234	296	SCL	6.4	13.2	-26.2	-3.4	722177	1861663
Don V-3	292	10YR5/1	369	311	320	CL	5.8	114.0	-26.0	-2.8	722352	1861433
Don V-4	292	10YR5/2	531	132	337	SC	5.8	34.2	-25.1	-0.5	722333	1861519
Don V-5	292	10YR6/2	308	319	373	CL	5.5	13.3	-26.3	-5.1	722090	1861487
Midden Str. A2-1	110	10YR4/2	416	212	373	CL	5.8	110.6	-23.8	-6.9	724239	1861726
Burial EK-03 A	0–10	10YR4/2	N.D.	N.D.	N.D.		6.7	9.5	-26.9	-4.0	722371	1861449
30 cm from bone	80–90	10YR6/4	N.D.	N.D.	N.D.		7.6	16.8				
Adjacent to the bone	85	10YR6/4	N.D.	N.D.	N.D.		7.4	127.0				

†L = Loam; CL = Clay Loam; SC = Sandy Clay; SCL = Sandy Clay Loam; N.D. = Not Determined

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