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Abstract

Millennial-scale biogeographic changes are well understood in many parts of the world, but little is known about long-term vegetation dynamics in subtropical regions. Here we investigate shifts in C$_3$/C$_4$ plants abundance occurred in central Argentina during the past few millennia. We determined present day soil organic matter δ^{13}C signatures of grasslands, shrublands and woodlands, containing different mixtures of C$_3$ and C$_4$ plants. We measured past changes in the relative cover of C$_3$/C$_4$ plants by comparing δ^{13}C values in soil profiles with present day δ^{13}C signatures. We analyzed ^{14}C activity in soil depths that showed major changes in vegetation. Present day relative cover of C$_3$/C$_4$ plants determines whole ecosystems' δ^{13}C signatures integrated as litter and superficial soil organic matter (R^2= 0.78; p<0.01). Deeper soils show a consistent shift in δ^{13}C, indicating a continuous replacement of C$_4$ by C$_3$ plants since 3,870 (±210) YBP. During this period, the relative abundance of C$_3$ plants increased 32% (average across sites) with significant changes being observed in all studied ecosystems. During the late Holocene C$_3$ plants became dominant in central Argentina. We identified increases in the relative C$_3$/C$_4$ cover in grasslands, shrublands and woodlands, suggesting a physiological basis for changes in vegetation. The replacement of C$_4$ by C$_3$ plants coincided with changes in climate towards colder and wetter conditions and could represent a shift in the C$_4$ species optimum range.

Keywords separated by ' - '

C$_3$/C$_4$ - Carbon isotopes - Climate change - Vegetation dynamics - SOM - Subtropics

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Evidence of shift in C_4 species range in central Argentina during the late Holocene

Lucas C. R. Silva · Melisa Giorgis · Madhur Anand · Lucas Enrico · Natalia Pérez-Harguindeguy · Valeria Falczuk · Larry L. Tieszen · Marcelo Cabido

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Abstract Millennial-scale biogeographic changes are well understood in many parts of the world, but little is known about long-term vegetation dynamics in subtropical regions. Here we investigate shifts in C_3/C_4 plants abundance occurred in central Argentina during the past few millennia. We determined present day soil organic matter $\delta^{13}C$ signatures of grasslands, shrublands and woodlands, containing different mixtures of C_3 and C_4 plants. We measured past changes in the relative cover of C_3/C_4 plants by comparing $\delta^{13}C$ values in soil profiles with present day $\delta^{13}C$ signatures. We analyzed ^{14}C activity in soil depths that showed major changes in vegetation. Present day relative cover of C_3/C_4 plants determines whole ecosystems' $\delta^{13}C$ signatures integrated as litter and superficial soil organic matter ($R^2=0.78$; $p<0.01$). Deeper soils show a consistent shift in $\delta^{13}C$, indicating a continuous replacement of C_4 by C_3 plants since 3,870 (±210) YBP. During this period, the relative abundance of C_3 plants increased 32% (average across sites) with significant changes being observed in all studied ecosystems. During the late Holocene C_3 plants became dominant in central Argentina. We identified increases in the relative C_3/C_4 cover in grasslands, shrublands and woodlands, suggesting a physiological basis for changes in vegetation. The replacement of C_4 by C_3 plants coincided with changes in climate towards colder and wetter conditions and could represent a shift in the C_4 species optimum range.

Keywords C_3/C_4 · Carbon isotopes · Climate change · Vegetation dynamics · SOM · Subtropics

Introduction

Paleovegetation reconstructions have shown that millennial-scale climate variability modulates the distribution of ecosystems, affecting global terrestrial biogeography. It is now clear from the palynological

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record that synchronic changes in plant species range occurred in the recent geological past as a response to climate change (Allen et al. 2010; Gajewski 2008; Williams et al. 2004). The vast majority of palynological studies, however, have been conducted in cold (mostly northern) regions and little is known about climatically driven vegetation dynamics in tropical and subtropical ecosystems. In South America, for example, palynological studies have been concentrated in cold zones of high altitudes, as unstable sedimentation, fast decay rates and exceedingly complex flora hinder the characterization of fossil pollen in warmer regions (Birks and Birks 2000; Flenley 1985; Marchant et al. 2002; Thouret et al. 1997). In areas where palynological investigation is possible, extrapolations from single isolated pollen profiles are typically used to infer regional shifts in vegetation (Behling and Pillar 2007; Ledru et al. 1996; Mancini 2009) and, for this reason, we still lack a detailed understanding of how past climate-vegetation interactions occurred.

Recent studies have successfully identified past vegetation changes in tropical and subtropical regions by analyzing soil organic carbon isotope ratios (Dümig et al. 2008; Silva et al. 2008, 2010a). Although more limited than palynological records for the purpose of describing changes in populations of individual species or genera, carbon isotopes can be used to trace paleo events at the ecosystem level. This technique is particularly useful to describe local-scale ecotonal shifts in predominant vegetation, where C_3- and C_4-dominated systems coexist. Generally, when light is not a limiting factor, plants with C_4 metabolism dominate warm environments, while C_3 plants predominate in cool climates (Sage 2004). The corollary is that C_4 outcompete C_3 plants in the tropics and subtropics, while the converse is observed in colder regions (Bond 2008). Fluctuations in climate, however, are expected to directly influence the balance between C_3 and C_4 plants, imposing alternate dominance of either metabolic pathway, explaining their co-occurrence through various scales of space and time.

When contrasting metabolic pathways also represent contrasting life forms (e.g. trees *versus* grasses), indirect effects of changes in climate may play a fundamental role on vegetation dynamics. For example, frequent and intense fires expected during warmer and drier periods would favor C_4 grasses at the expense of C_3 woody plants in local scales (Behling et al. 2004; Hoffmann et al. 2003). On the other hand, high atmospheric CO_2 levels globally, could favor C_3 species because of their lower photosynthetic efficiency when compared with C_4 plants (Epstein et al. 1997; Luo et al. 2006; Sage et al. 2010). Recent studies have reported the expansion of C_3- (forests) over C_4-dominated ecosystems (savannas and grasslands) during the late Holocene (Behling et al. 2005, 2004; Dümig et al. 2008; Silva et al. 2008). Unfortunately, these studies were conducted where distinct metabolisms represent differences in life form and for this reason they were not able to disentangle direct and indirect effects of climate fluctuations on vegetation change.

Here we further investigate this issue, asking whether Holocene climate change has had a significant direct effect on terrestrial ecosystems, promoting an overall competitive advantage of C_3 over C_4 plants, independently of differences in species life form. To answer this question we sampled a broad region in central Argentina, which corresponds to the present day southernmost limit of C_4 grasses distribution within the Americas (Ehleringer et al. 2005). We investigated ecosystems of different structures (grassland, shrubland and woodland) containing various proportions of C_3 and C_4 plants. We quantitatively described shifts on C_3/C_4 vegetation cover occurred in the past using isotopic analysis of organic carbon and discussed the results in relation to previously reported climate and vegetation reconstructions.

Material and methods

Study region

The present study was conducted in central Argentina, within the limits of Córdoba province (Fig. 1). Both C_4 (grasses) and C_3 (herbaceous and/or woody) plants coexist in the five major regional phytogeographical units: Pampa, Espinal, Chaco, Monte and Pastizal de altura (Cabrera 1976). These distinct vegetation types represent a gradient that encompasses grasslands to the eastern lowlands (~100 m ASL), woodlands towards west at intermediate altitudes (300–500 m), xerophytic open shrublands in the semi-arid western highlands (800–1,200 m) and grasslands in higher altitudes (above the tree line, 1,600 m) (Fig. 1).

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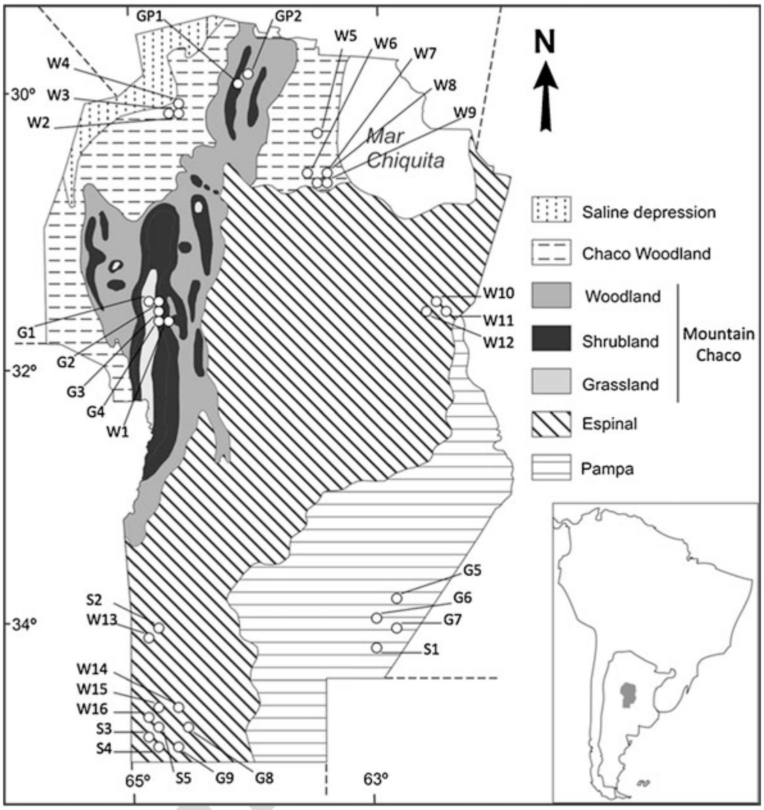

Fig. 1 The vegetation of Cordoba as represented by the most important phytogeographical provinces. Circles show the approximated location of the study sites, where W; S and G and GP represent woodland; shrubland, grassland, and grassland with palms respectively

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According to the Köppen-Geiger classification, the regional climate is temperate/warm-temperate to subtropical, in average ranging from 10°C to 24°C between winter and summer (summer-time highs ~30°C and winter lows ~4°C), with a marked rainfall gradient from east (annual rainfall >1,000 mm) to west (annual rainfall <400 mm), with 70% of the rainfall occurring from November to March (Cabido et al. 2008).

Vegetation survey

The regional landscape has been severely altered by human activity throughout the past centuries, but relicts of undisturbed vegetation can still be found. Using our own unpublished and other authors' published recent floristic surveys (Cabido et al. 1997, 1993, 2008; Diaz et al. 1994, 1999, 2001; Perez-Harguindeguy et al. 2000; Pucheta et al. 1998; Zak and Cabido 2002), we selected 32 well-preserved sites where the vegetation is representative of the most important regional ecosystems. We classified the distinct vegetation types in four major categories: woodlands (16 sites); shrublands (5 sites); grasslands (9 sites) and grasslands with palms (2 sites) (W, S, G and GP sites indicated in Fig. 3). The number of sites representing each of these vegetation categories varied according to their floristic complexity. Woodlands comprise both xerophytic and mesophytic ecosystems with high diversity of woody and non-woody plants, dominated by *Aspidosperma* and species of *Prosopis*, while shrublands only represent xerophytic ecosystems dominated by species of *Geoffroea*, *Condalia* or *Maytenus* genera. Grasslands encompass both C_3- and C_4-dominated treeless vegetation. In a couple of grassland sites the presence of palms of the species *Trithrinax campestris* was remarkable and for this reason these sites were classified as grassland with palms.

In previous studies we used leaf area index (LAI) measurements to characterize C_3/C_4 relative cover across vegetation gradients (see for example: Silva et al. 2008, 2010a, b, c). These studies were conducted, however, in regions where different strata represent distinct metabolic pathways (e.g. herbaceous layer is dominated by C_4 grasses, while shrubs and trees are

C_3). In such conditions, LAI measurements (which do not differentiate between species) are sufficient to describe changes in the relative cover of C_3 and C_4 plants. On the contrary, in the region studied here perennial C_3 and C_4 herbaceous plants can be equally abundant (Zuloaga et al. 2008) and predominant metabolic pathways can only be assessed by detailed floristic surveys. For this reason, we performed a complete census, including all herbaceous, shrub and tree species at each studied site. Our survey was conducted during the growing season (Summer) of 2010, when maximum productivity is typically attained and all potential species are present. The relative contribution of each species for the total vegetation cover was estimated (visual estimation—projection of canopy cover in 400 m^2 plots—3 plots per site) and from these estimates the relative cover of C_3 and C_4 species was calculated (Cabido et al. 1997, 2008; Renison et al. 2006). Plants that have the C_4 photosynthetic pathway were distinguished from those that have the C_3 pathway by examination of the Kranz anatomy in cross-sections of fresh and herbarium specimens and from the literature (Sánchez and Arriaga 1990; Smith and Epstein 1971). A list of the surveyed species, including growth habit and metabolic pathways, is presented in Appendix 1. More details about the regional vascular flora can be found at Zuloaga et al. (2008), but see also their on line updates at: www.darwin.edu.ar.

Isotopic signatures and past vegetation changes

To test whether and how much C_3 and C_4 relative vegetation cover has changed we relied on two well-known natural processes: (i) during photosynthesis C_3 plants discriminate more against CO_2 molecules that contain the stable isotope ^{13}C than do C_4 plants. For this reason different proportions of C_3/C_4 contribution to the total biomass yield differences in the mean carbon isotopic ratios ($\delta^{13}C$) in the soil organic matter (SOM) (Ehleringer et al. 2000; Marshall et al. 2007; Silva et al. 2010a, b, 2008; Smith and Epstein 1971; Von Fischer et al. 2008); (ii) SOM in soil profiles represents a chronological sequence of vegetation signature, with past vegetation recorded at deeper levels (Dümig et al. 2008; Nordt et al. 2007; Sanaiotti et al. 2002; Silva et al. 2010a, 2008; Victoria et al. 1995). Based on these we characterized past shifts in vegetation structure by, firstly, determining the current vegetation cover at each study site (described in the previous section), secondly, by determining the carbon isotopic signatures ($\delta^{13}C$) in the litter and superficial SOM and, finally, by examining the $\delta^{13}C$ of SOM at different depths in soil profiles.

We used 3 to 5 soil profiles at each site to sample: fresh litter (any decaying organic matter found at the uppermost soil layer), superficial (0–1 cm depth) and deep soil (10, 20, 30, 50 and 100 cm depth). Prior to analysis litter was dried for two days at 70°C and coarsely ground, while superficial and deep soils were dried at room temperature after which fine roots were removed by sieving through a 0.8 mm mesh and not analyzed. The carbon isotope ratios of litter and soil samples were determined at the Laboratory of Stable Isotope Ecology (LSIETE) at the University of Miami. Samples (10 mg) were loaded in tin cups, which were placed in an automated elemental analyzer connected to a continuous flow isotope ratio mass spectrometer. ^{13}C abundances were expressed as:

$$\delta^{13}C\ (‰) = (R_{sample}/RPDB\text{-}1) \times 1000$$

where R_{sample} and RPDB represent the $^{13}C/^{12}C$ ratios and PeeDee standard respectively. The precision of analysis was ±0.1‰ (±SD).

We used regression analysis to describe the effect of present day C_3/C_4 relative vegetation cover on litter and superficial SOM $\delta^{13}C$ values (signatures). Based on the equation that best described this relationship (Fig. 2) we estimated past C_3/C_4 relative cover, by

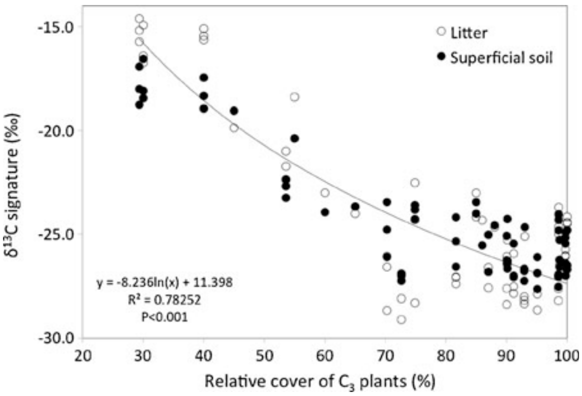

Fig. 2 Relationship between the relative vegetation cover of C_3 plants and the isotopic composition ($\delta^{13}C$) of litter and superficial soil organic matter (0–1 cm depth), across all study sites

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examining SOM $\delta^{13}C$ signatures at deeper SOM in soil profiles. We estimated past vegetation cover for each study site and soil depth (1, 10, 20, 30, 50 and 100 cm). By calculating percent differences between the C_3/C_4 relative cover estimated in superficial (0–1 cm) and in deep soils, where major shifts in vegetation were observed (50 cm), we determined the total vegetation change. We also measured the slope (angular coefficient) of least square regressions, performed between estimates of C_3/C_4 cover and their respective soil depth at each study site. All soil depths were used for this calculation, here named degree of vegetation change. We used two-way analysis of variance (ANOVA) to test the effect of ecosystem, altitude and their interaction on both metrics of vegetation change (total percent and degree of vegetation change) across sites. Analysis of variance and regression analysis were performed according to standard methods, using the software JMP Version 9 (JMP 2009). For brevity, only statistically significant results are presented.

Carbon dating

We analyzed ^{14}C activity of carbon in the SOM of the soil profiles that showed major shifts in C_3/C_4 vegetation cover at each ecosystem (grassland, shrubland and woodland: G4, G8, GP1, S1, W7 and W13; all at 50 cm depth; Fig. 1). Because roots from the modern day vegetation can exude recent ^{14}C into the deeper soil matrix, dates acquired by analyzing SOM ^{14}C may be underestimated. For this reason, SOM ^{14}C measurements should be considered as a proxy for determining the minimum possible date of past vegetation changes (Dümig et al. 2008; Martinelli et al. 1996; Silva et al. 2010a, 2008; Trumbore 2000; Victoria et al. 1995). Analyses of ^{14}C activity were performed using accelerator mass spectrometry at the Beta Analytic Radiocarbon Dating Laboratory (Miami, USA). The results were then calibrated to represent actual calendar years of the mean age of SOM. The calibration was performed using the newest (2004) calibration database (Reimer et al. 2004). Calibrated dates expressed as years before present (YBP) were consistent among sites/ecosystems and here we present the average date and standard deviation of measurements that represent major vegetation shifts across sites.

Results

Current vegetation cover and $\delta^{13}C$ signatures

Differences in the present day relative contribution of C_3 and C_4 plants to total vegetation cover explain variations in whole ecosystems' $\delta^{13}C$ signatures, integrated as litter and superficial soil organic matter ($R^2=0.78$; $p<0.01$) (Fig. 2). Similar relationships have been found in previous studies conducted in different ecosystems (Lloyd et al. 2008; Silva et al. 2010a, 2008) and represent an expected gradient determined by variations in the amount of C_3- and C_4-originated biomass and their mixture. Due to intrinsic differences in discrimination, C_3 plants $\delta^{13}C$ signature (ranging from −20 to −35‰) are unmistakably distinguishable from C_4 plants $\delta^{13}C$ signature (ranging from −9 to −15‰) (Ehleringer et al. 1993, 1997). The $\delta^{13}C$ values found here ranged from −15‰ in ecosystems dominated by C_4 grasses to −29‰ in ecosystems dominated by C_3 plants (either herbaceous or woody plants). This supports our estimates of vegetation cover as being representative of the total in-situ biomass production, attributable to either C_3 or C_4 metabolic pathways. Both litter and superficial SOM $\delta^{13}C$ significantly reflected the current vegetation cover and, thus, could be used as reference to trace past changes in vegetation recorded in the SOM at deeper soil layers.

Past vegetation change

Using the equation presented on Fig. 2 and isotopic measurements performed at different depths of soil profiles, we calculated changes in the relative C_3 and C_4 vegetation cover at each study site. Our results show a consistent signal of continuous vegetation change, with C_4 being replaced by C_3 plants. Measurements of ^{14}C activity show that this process began approximately 3,870 (±210) years before present and, since then, the relative abundance of C_3 plants increased 32% in average (Fig. 3). All woodlands and shrublands, currently dominated by C_3 plants (> than 70% of the total vegetation cover), had a greater C_4 cover in the past. In some cases C_4 grasses were the predominant vegetation, while C_3 plants represented less than 20% of the total vegetation cover (see sites W1, W9, W13; Fig. 3). The least noticeable shift observed in woodlands corresponds to

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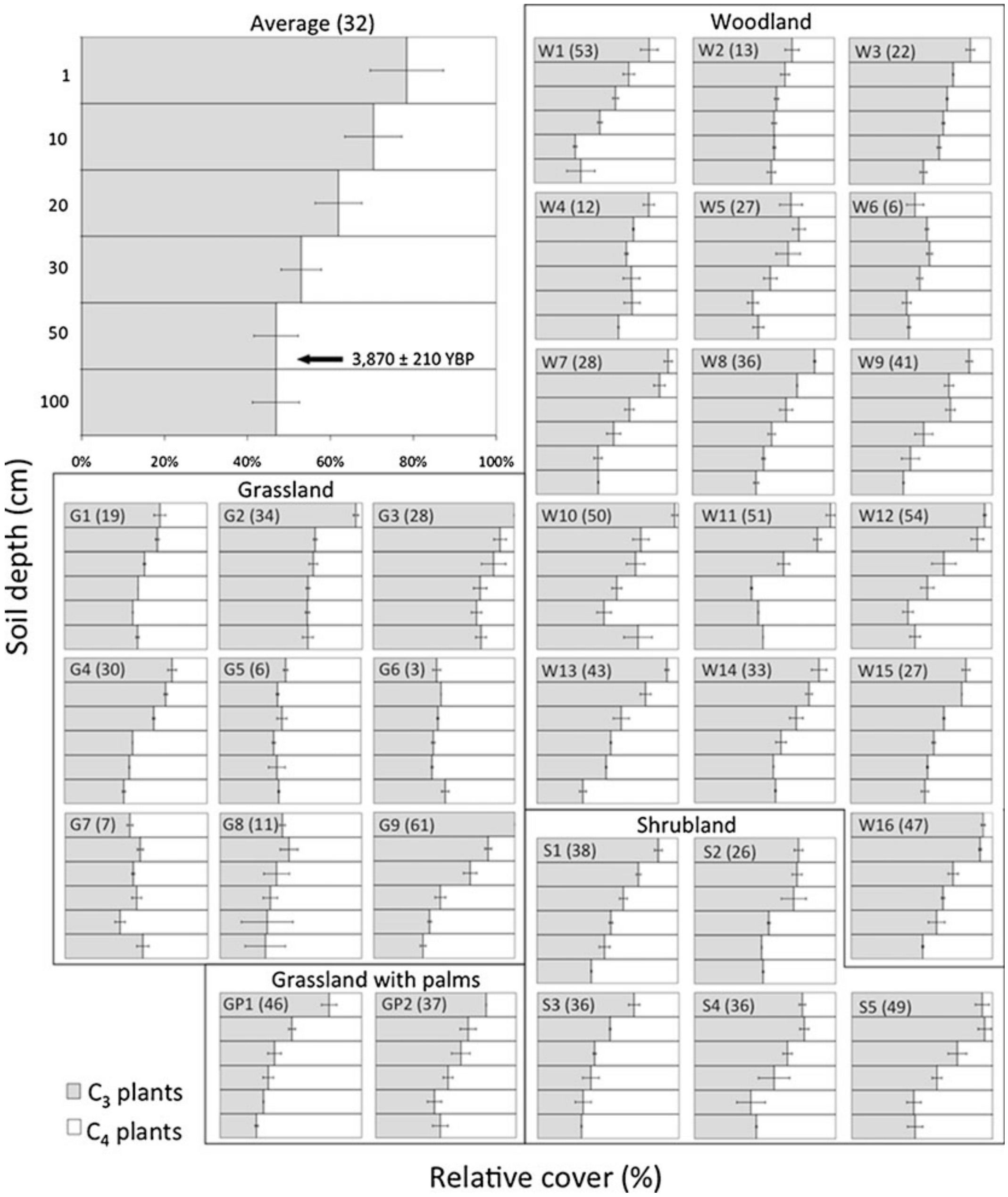

Fig. 3 Changes in the relative cover of C_3 and C_4 plants, estimated from $\delta^{13}C$ values measured in the soil organic matter (SOM) at different depths of soil profiles. The conversion of $\delta^{13}C$ values into percent cover followed the relationship found between present day vegetation cover and $\delta^{13}C$ signatures measured in the litter and superficial SOM (Fig. 2). Grey and white bars represent average values of C_3 and C_4 cover at each study site (3 to 5 profiles per site) and error bars correspond to one standard deviation. Numbers in brackets show total percent change in vegetation cover since 3,870 (±210) years before present. Site locations are shown on Fig. 1

a 6% change towards a greater C_3 cover in the present (W6; Fig. 3). In other woodland sites C_3 cover increased at least 12%, often reaching values greater than 40%. Similarly, the replacement of C_4 by C_3 plants in shrublands ranged from 26 to 49% in the past few thousand years (Fig. 3). In grasslands, vegetation cover remained nearly unaltered in a couple of sites (see G6, G7; Fig. 3), but in most cases we observed shifts in C_3/C_4 cover of the same magnitude (in some cases higher) than those observed in shrub and woodland sites (see for example G9= 61%; Fig. 3).

It is important to note that these results only represent records of past vegetation at each given site. However, the fact that expressive changes in vegetation occurred in most sites and always in the same direction (C_3 replacing C_4 plants), suggests a regional scale shifts in vegetation. Two-way ANOVA models indicate that changes in vegetation, measured as percent differences between past and recent C_3/C_4 cover, occurred independently of ecosystem (grassland, shrubland and woodland), altitude (sites ranged from 100 to 2,012 m asl) or their interaction. Likewise, the degree of vegetation change across sites, measured as the angular coefficient of least square regressions between C_3/C_4 cover and soil depths, were not affected by ecosystem, altitude or their interaction (Appendix 2).

Discussion

$\delta^{13}C$ signatures and past vegetation change

We found a highly significant relationship between the relative cover of C_3 (herbaceous and woody) and C_4 (grasses) with $\delta^{13}C$ values measured in the organic matter across sites. It has been well documented that differences in the total contribution of C_3- and C_4-originated biomass to whole ecosystems production yield distinguishable carbon isotopic signatures (Dümig et al. 2008; Ehleringer et al. 2000; Lloyd et al. 2008; Sanaiotti et al. 2002; Silva et al. 2010a, b, 2008; Victoria et al. 1995; Von Fischer et al. 2008). Similarly, here we show that differences in vegetation cover, with respect to metabolic pathways, explain variations in soil organic matter $\delta^{13}C$ signatures. Litter carbon isotope ratios varied with C_3/C_4 cover, remaining nearly unaltered after decomposition. The spectrum of $\delta^{13}C$ ratios reported here, varying up to 15‰ depending on the vegetation cover, is consistent with those described in different C_3- and C_4-dominated ecosystems (Lloyd et al 2008, Silva et al 2008, Von Fischer et al. 2008) and represent reliable signatures that can be used to trace shifts in vegetation through time.

Although processes other than changes in vegetation may alter isotopic ratios before and after litter deposition, major paleoecological events can be identified with good confidence in soil profiles, because organic matter $\delta^{13}C$ ratios tend to be stable long after decomposition, conserving vegetation signatures throughout millennia (Silva et al. 2008; Von Fischer et al. 2008). Changes in the isotopic composition of atmospheric CO_2 could have affected values of $\delta^{13}C$ prior to deposition, but with the exception of past century, $\delta^{13}C$ variations in atmospheric CO_2 have been small (<1.0‰ within the last 10,000 years; Flückiger et al. 2002). Variations in $\delta^{13}C$ due to changes in photosynthetic discrimination are also possible, but would not have produced similar changes across sites/ecosystems and even if so, such variations would have been more than one order of magnitude smaller than the range observed here (Martin-Benito et al. 2010; Nock et al. 2010; Silva et al. 2010c, 2009).

Differences in root depth between trees and grasses could have contributed to soil organic matter isotopic enrichment with depth. However, the presumably deeper rooting habit of trees could have depleted (C_3 signal), but not enriched (C_4 signal), SOM $\delta^{13}C$. Moreover, differences in root depth between C_3 and C_4 plants typically yield small changes in $\delta^{13}C$ and isotopic offsets that exceed 3‰ are usually interpreted as a shift in vegetation (Krull et al. 2002; Von Fischer et al. 2008). Additionally, because changes in vegetation were observed in different ecosystems (grassland, shrubland and woodland), differences in root depth cannot explain changes in $\delta^{13}C$ observed here. Post depositional differential degradation of chemical compounds and fractionation associated with microbial activity could also have affected soil organic matter isotopic ratios (Marshall et al. 2007). However, this influence is typically observed only within the top 20 cm of the soil profile, correlated with total soil organic carbon content (Ehleringer et al. 2000; Krull et al. 2002). We observed changes in $\delta^{13}C$ values at much deeper layers of the soil profile that were not correlated with soil carbon content. Total soil carbon in the study region range from 2 to 26 gkg^{-1} according to vegetation type and altitudinal/climatic variations (Alvarez and Lavado 1998), yet we observed consistent variations in $\delta^{13}C$ across sites, which did not change significantly in different ecosystems or altitudes. For these reasons, we conclude that changes in $\delta^{13}C$ values observed with soil depth in the present study were caused by past changes in vegetation.

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A possible northward shift in the C_4 species range

We detected a convergent signal of soil organic matter isotopic signatures, showing a continuous increase in the relative abundance of C_3 plants since 3,870 (±210) years ago. Previous palynological and isotopic studies from southern South America have identified the expansion of C_3-dominated (forests) over C_4-dominated ecosystems (savannas and grasslands) during the same period (Behling et al. 2005, 2004; Dümig et al. 2008; Mancini 2009; Silva et al. 2008; Wille and Schabitz 2009). While these studies have described local shifts in forest-savanna or forest-grassland ecotones, the results presented here show that vegetation changed at a regional scale and independently of ecosystem structure. In the past, C_4 grasses greatly contributed to the total biomass of sites now dominated by distinct groups of C_3 plants. Because changes in the predominant metabolic pathway occurred in woodlands, shrublands and grasslands, they were probably not related to intrinsic differences in plant life forms but rather had a physiological basis.

Changes in ecosystem structure, from dense to open vegetation, can be site-specific and determined by local environmental gradient or disturbance history (Crisci et al. 1991; Da Silva and Bates 2002). For example, it has been shown that spatial and/or temporal changes in vegetation structure may be attributed to variations in edaphic properties (Haridasan 2008), water availability (Bush et al. 2004; Furley et al. 1992; Silva et al. 2009), fire regime (Accatino et al. 2010; Hoffmann et al. 2003; Moreira 2000) and grazing intensity (Diaz et al. 2001). We suspect that all these factors have contributed to configure the current structure of the vegetation at each studied site. However, a simultaneous shift in the predominant metabolic pathway across sites, suggests that a major force has promoted changes in plant communities during the late Holocene.

We believe that changes in climate were responsible for the shift in vegetation reported here. Worldwide, the extant C_4 genera occupy a wider range of drier and warmer habitats than their C_3 counterparts because the C_4 pathway represents a pre-adaptation to hot and arid conditions (Osborne and Freckleton 2009). Reconstructions of paleoclimate have shown a significant drop in global temperatures during the past few thousand years (Fig. 4). This climate cooling followed the retreat of glaciers in the northern hemisphere during the Holocene climatic optimum, which also led to substantial water input in tropical and subtropical systems (Markgraf et al. 1992; Vimeux et al. 2009; Wanner et al. 2008). Fossil pollen, charcoal and isotopic evidence from different South American biomes indicate that C_3 plants became more abundant during the late Holocene, coinciding with changes in climate towards colder and wetter conditions (Behling et al. 2005, 2004, Mancini 2009, Silva et al. 2008).

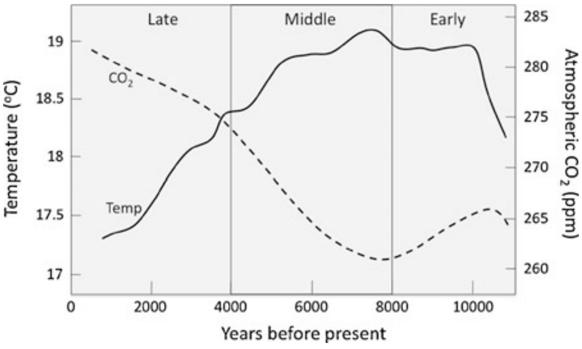

Fig. 4 Temperature and CO_2 records for the Holocene time period. Temperature record according to alkenone measurements (core GeoB 7139–2) of north-central Chile (De Pol-Holz et al. 2006) and CO_2 record described from ice core analyses (EPICA ice cores; Flückiger et al. 2002)

Presently, the composition of C_3 and C_4 species in the study region follow temperature parameters more strongly than rainfall (Cavagnaro 1988, Cabido Q2 1997). Along altitudinal gradients C_4 appear in greater number than C_3 species in locations where

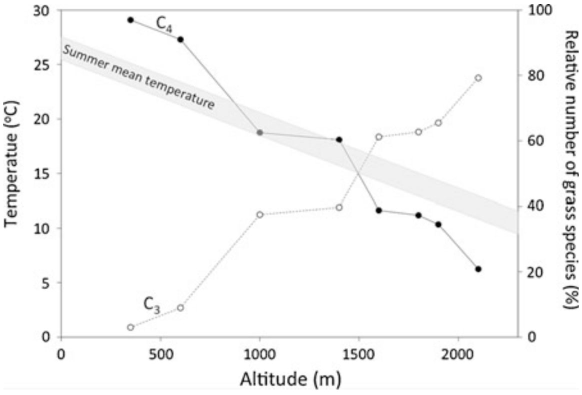

Fig. 5 Changes in the present day relative species composition of C_3 and C_4 grasses across an altitudinal gradient in central Argentina, as defined by summer (January) average temperatures (adapted from Cabido et al. 1997)

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summers are warmer than 18°C, while in colder areas the number of C_3 species represent more than 60% of the total grass diversity (Fig. 5). Although the number of species is not necessarily linked to the total cover of either C_3 or C_4 plants, it seems fair to assume that colder temperatures (average annual temperatures dropped from >18.5°C to <17.5°C; Fig. 4) increased the competitive advantage and, consequently, the relative abundance of C_3 plants at the latitude studied here. Increases in atmospheric CO_2 concentrations could also have favored C_3 at the expenses of C_4 plants (Epstein et al. 1997; Huang et al. 2007; Luo et al. 2006; Sage et al. 2010). Atmospheric CO_2 levels have increased monotonically (~20 ppm in total) since the mid Holocene (Fig. 4). The reasons are debatable, but decreases in total terrestrial biomass in response to colder temperatures (Indermühle et al. 1999) and a drop in oceanic carbonate ion concentration (Broecker et al. 2001) are possible causes behind this natural CO_2 enrichment. However, even though C_3 plants are more responsive to elevations in CO_2 than C_4 plants, enrichments of this magnitude should not have altered the competitive balance between these two metabolic pathways (Wand et al. 1999). Therefore, changes in temperature are the most likely explanation for a regional scale vegetation displacement.

Despite increasingly higher atmospheric CO_2 levels, warmer global temperatures are expected to promote the expansion of C_4 species range in the near future (Sage 2004; Sage et al. 2010; Wand et al. 1999). This could represent a return to C_4-dominated systems in the latitude studied here, as observed during warmer periods. It is important to note however that the velocity of climate change during the Holocene allowed time for species to migrate in locked step with their optimal range and the consequences of the much faster rates of recent global warming on plant communities remain unknown. There is still great uncertainty in predicting the future of C_3- and C_4-dominated ecosystems globally (Bond 2008). So far attempts to predict climate change impacts on terrestrial ecosystems have been limited to correlations between the current climate and species distribution (Pearson and Dawson 2003). The search for long-term convergent patterns as those described here, could lead to an improved picture of climate-vegetation interactions, increasing our ability to predict the future impacts of climate change worldwide.

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Appendix 1

Table 1 Identity, life form and metabolic pathway of all plant species present in the studied sites. Plants with C_4 photosynthetic pathway were distinguished from those that have the C_3 pathway by examination of the Kranz anatomy in cross-sections of fresh and herbarium specimens and from the literature

Family/species	Life form	Metabolic pathway
Acanthaceae		
Dicliptera squarrosa Nees	herb	C3
Justicia squarrosa Griseb.	herb	C3
Stenandrium dulce (Cav.) Nees	herb	C3
Amaranthaceae		
Amaranthus hybridus L. ssp. hybridus	herb	C3
Guilleminea densa (Willd. ex Roem. & Schult.) Moq.	herb	C3
Iresine diffusa Humb. & Bonpl. ex Willd. var. diffusa	herb	C3
Pfaffia gnaphaliodes (L. f.) Mart.	herb	C3

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t1.12 **Table 1** (continued)

	Family/species	Life form	Metabolic pathway
t1.13	Amaryllidaceae		
t1.14	Zephyranthes longistyla Pax	herb	C3
t1.15	Anacardiaceae		
t1.16	Lithraea molleoides (Vell.) Engl.	tree	C3
t1.17	Schinus bumeloides I.M. Johnst.	shrub	C3
t1.18	Schinus fasciculatus (Griseb.) I. M. Johnst. var. fasciculatus	shrub	C3
t1.19	Apiaceae		
t1.20	Ammi visnaga (L.) Lam.	herb	C3
t1.21	Bowlesia incana Ruiz & Pav.	herb	C3
t1.22	Conium maculatum L.	herb	C3
t1.23	Eryngium agavifolium Griseb.	herb	C3
t1.24	Eryngium horridum Malme	herb	C3
t1.25	Eryngium nudicaule Lam.	herb	C3
t1.26	Apocynaceae		
t1.27	Amblyopetalum coccineum (Griseb.) Malme	herb	C3
t1.28	Aspidosperma quebracho-blanco Schltdl.	tree	C3
t1.29	Ditassa buchellii Hook. & Arn.	epiphyte	C3
t1.30	Metastelma tubatum Griseb.	epiphyte	C3
t1.31	Morreria odorata (Hook. & Arn.) Lindl.	epiphyte	C3
t1.32	Arecaceae		
t1.33	Trithinax campestris (Burmeist.) Drude & Griseb.	palm	C3
t1.34	Asteraceae		
t1.35	Ambrosia tenuifolia Spreng.	herb	C3
t1.36	Baccharis coridifolia DC.	herb	C3
t1.37	Baccharis pingraea DC.	herb	C3
t1.38	Baccharis salicifolia (Ruiz & Pav.) Pers.	herb	C3
t1.39	Baccharis stenophylla Ariza	herb	C3
t1.40	Baccharis ulicina Hook. & Arn.	herb	C3
t1.41	Bidens andicola var. decomposita Kuntze	herb	C3
t1.42	Bidens pilosa L. var. pilosa	herb	C3
t1.43	Bidens subalternans DC.	herb	C3
t1.44	Carduus acanthoides L.	herb	C3
t1.45	Carduus thoermeri Weinm.	herb	C3
t1.46	Chaptalia nutans (L.) Pol.	herb	C3
t1.47	Cirsium vulgare (Savi) Ten.	herb	C3
t1.48	Conyza bonaeriensis (L.) Cronquist	herb	C3
t1.49	Conyza primulifolia (Lam.) Cuatrec. & Lourteig	herb	C3
t1.50	Eupatorium inulifolium Kunth	herb	C3
t1.51	Eupatorium patens D. Don ex Hook. & Arn.	herb	C3
t1.52	Eupatorium viscidum Hook. & Arn.	herb	C3
t1.53	Galinsoga parviflora Cav.	herb	C3
t1.54	Gamochaeta falcata (Lam.) Cabrera	herb	C3
t1.55	Gamochaeta sp.	herb	C3
t1.56	Gnaphalium gaudichardianum DC.	herb	C3
t1.57	Heterosperma ovatifolium Cav.	herb	C3

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Table 1 (continued)

Family/species	Life form	Metabolic pathway
Heterotheca subaxillaris (Lam.) Britton & Rusby	herb	C3
Hypochaeris caespitosa Cabrera	herb	C3
Lactuca serriola L.	herb	C3
Schkuria pinnata (Lam.) Kuntze ex Thell.	herb	C3
Senecio ceratophylloides Griseb.	herb	C3
Senecio pampeanus Cabrera	herb	C3
Sonchus oleraceus L.	herb	C3
Synedrellopsis grisebachii Hieron. & Kuntze	herb	C3
Tagetes minuta L.	herb	C3
Taraxacum officinale G. Weber ex F. H. Wigg.	herb	C3
Verbesina encelioides (Cav.) Benth. & Hook. f. ex A. Gray	herb	C3
Vernonia incana Less.	herb	C3
Vernonia nudiflora Less.	herb	C3
Xanthium spinosum L. var. spinosum	herb	C3
Basellaceae		
Anredera cordifolia (Ten.) Steenis	epiphyte	C3
Bignoniaceae		
Amphilophium cynanchoides (DC.) L. G. Lohmann	epiphyte	C3
Dolichandra cynanchoides Cham.	epiphyte	C3
Brassicaceae		
Descurainia argentina O.E. Schulz	herb	C3
Exhalimolobos weddellii (E. Fourn.) Al-Shehbaz & C. D. Bailey	herb	C3
Lepidium bonariense L.	shrub	C3
Bromeliaceae		
Tillandsia aizoides Mez	epiphyte	C3
Tillandsia bryoides Griseb. ex Baker	epiphyte	C3
Tillandsia capillaris Ruiz & Pav.	epiphyte	C3
Tillandsia duratii Vis. var. duratii	epiphyte	C3
Tillandsia xiphioides Ker Gawl. var. xiphioides	epiphyte	C3
Calyceraceae		
Boopis anthemoides Juss.	herb	C3
Campanulaceae		
Wahlenbergia linarioides (Lam.) A. DC.	herb	C3
Capparaceae		
Capparis atamisquea Kuntze	shrub	C3
Caryophyllaceae		
Cardionema ramosissima (Weinm.) A. Nelson & J. F. Macbr.	epiphyte	C3
Polycarpon tetraphyllum (L.) L.	herb	C3
Silene antirrhina L. var. antirrhina	herb	C3
Celastraceae		
Moya spinosa Griseb.	shrub	C3
Celtidaceae		
Celtis ehrenbergiana (Klotzch) Liebm.	tree	C3
Chenopodiaceae		

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Table 1 (continued)

Family/species	Life form	Metabolic pathway
Allenrolfea patagonica (Moq.) Kuntze	herb	C3
Atriplex lampa (Moq.) D. Dietr.	herb	C3
Chenopodiaceae sp.	herb	C3
Chenopodium album L.	herb	C3
Salsola kali L.	herb	C3
Sarcocornia ambigua (Michx.) M.A. Alonso & M.B. Crespo	herb	C3
Commelinaceae		
Commelina erecta L.	herb	C3
Convolvulaceae		
Cressa truxillensis Kunth	herb	C3
Dichondra microcalyx (Hallier f.) Fabris	herb	C3
Evolvulus sericeus Sw.	herb	C3
Ipomoea hieronymi (Kuntze) O'Donell	epiphyte	C3
Ipomoea nil (L.) Roth	epiphyte	C3
Ipomoea sp.	epiphyte	C3
Cucurbitaceae		
Sicyos malvifolius Griseb.	epiphyte	C3
Cyperaceae		
Bulbostylis juncoides (Vahl) Kük. ex Herter var. juncoides	sedge	C3
Carex sororia Kunth ssp. sororia	sedge	C3
Cyperacee sp.	sedge	C3
Cyperus aggregatus (Willd.) Endl. var. aggregatus	sedge	C3
Cyperus entrerianus Boeck.	sedge	C3
Cyperus hermaphroditus (Jacq.) Standl.	sedge	C3
Cyperus incomtus Kunth	sedge	C3
Ephedraceae		
Ephedra americana Humb. & Bonpl. ex Willd.	epiphyte	C3
Ephedra triandra Tul. emend. J. H. Hunz.	epiphyte	C3
Euphorbiaceae		
Acalypha communis Müll. Arg.	herb	C3
Croton lachnostachyus Baill.	herb	C3
Euphorbia acerensis Boiss.	herb	C3
Euphorbia dentata Michx.	herb	C3
Euphorbia lorentzii Müll. Arg.	herb	C3
Euphorbia serpens Kunth	herb	C3
Tragia geraniifolia Klotzch ex Baill.	herb	C3
Tragia hieronymii Pax & K. Hoffm.	herb	C3
Fabaceae		
Acacia aroma Gillies ex Hook & Arn.	shrub	C3
Acacia caven (Molina) Molina	shrub	C3
Acacia gilliesii Steud.	shrub	C3
Acacia praecox Griseb.	shrub	C3
Adesmia bicolor (Poir.) DC.	epiphyte	C3
Cercidium praecox (Ruiz & Pav. ex Hook.) Harms glaucum Ssp. (Cav.) Burkart & Carter	tree	C3

Table 1 (continued)

Family/species	Life form	Metabolic pathway
Cologamia broussonetii (Balb.) DC.	epiphyte	C3
Coursetia hassleri Chodat	herb	C3
Desmanthus sp.	herb	C3
Desmodium uncinatum (Jacq.) DC.	herb	C3
Fabaceae sp.	herb	C3
Geoffraea decorticans (Gillies ex Hook. & Arn.) Burkart	tree	C3
Gleditsia triacanthos L.	tree	C3
Medicago lupulina L.	herb	C3
Mimosa detinens Benth.	shrub	C3
Mimozyganthus carinatus (Griseb.) Burkart	shrub	C3
Prosopis alba Griseb.	tree	C3
Prosopis caldenia Burkart	tree	C3
Prosopis flexuosa DC.	tree	C3
Prosopis nigra (Griseb.) Hieron. var. nigra	tree	C3
Prosopis pugionata Burkart	tree	C3
Prosopis torquata (Cav. ex Lag.) DC.	tree	C3
Rhynchosia senna Griseb. ex Hook.	epiphyte	C3
Senna aphylla (Cav.) H. S. Irwin & Barneby	herb	C3
Geraniaceae		
Geranium dissectum L.	herb	C3
Juncaceae		
Juncus acutus L.	sedge	C3
Juncus imbricatus Laharpe	sedge	C3
Juncus sp	sedge	C3
Lamiaceae		
Clinopodium gilliesii (Benth.) Kuntze	shrub	C3
Hyptis floribunda (Briq.) Briq. ex Micheli	herb	C3
Hyptis mutabilis (Rich.) Briq.	herb	C3
Leonurus japonicus Houtt.	herb	C3
Lythraceae		
Cuphea glutinosa Cham. & Schltdl.	herb	C3
Heimia salicifolia (Kunth) Link	herb	C3
Malpighiaceae		
Cordobia argentea (Griseb.) Nied.	epiphyte	C3
Janusia guaranitica (A. St.-Hil.) A. Juss.	epiphyte	C3
Tricomaria usillo Hook. & Arn.	shrub	C3
Malvaceae		
Krapovicasea flavescens	herb	C3
Malvastrum coromandelianum (L.) Garcke	herb	C3
Modiolastrum malvifolium (Griseb.) K. Schum.	herb	C3
Pseudoabutilon pedunculatum (R. E. Fr.) Krapov.	herb	C3
Sida dictyocarpa Griseb. ex K. Schum.	herb	C3
Sida rhombifolia L.	herb	C3
Sida spinosa L.	herb	C3

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Table 1 (continued)

Family/species	Life form	Metabolic pathway
Moraceae		
Morus alba L.	tree	C3
Olacaceae		
Ximenia americana L.	shrub	C3
Onagraceae		
Oenothera affinis Cambess.	herb	C3
Oenothera sp.	herb	C3
Orchidaceae		
Cyclopogon elatus (Sw.) Schltr.	herb	C3
Habenaria sp.	herb	C3
Oxalidaceae		
Oxalis conorrhiza Jacq.	herb	C3
Passifloraceae		
Passiflora mooreana Hook. f.	epiphyte	C3
Phytolaccaceae		
Petiveria alliacea L.	herb	C3
Rivinia humilis L.	herb	C3
Plantaginaceae		
Plantago tomentosa Lam.	herb	C3
Poaceae		
Agrostis montevidensis Spreng. ex Nees f. montevidensis	grass	C3
Aristida laevis (Nees) Kunth	grass	C4
Aristida mendocina Phil.	grass	C4
Bothriochloa barbinodis (Lag.) Herter	grass	C4
Bothriochloa laguroides (DC.) Herter ssp. laguroides	grass	C4
Bothriochloa springfieldii (Gould) Parodi	grass	C4
Bromus catharticus Vahl var. catharticus	grass	C3
Cenchrus myosuroides Kunth var. myosuroides	grass	C4
Cenchrus spinifex Cav.	grass	C4
Chascolytrum subaristatum (Lam.) Desv.	grass	C3
Chloris halophila Parodi var. halophila	grass	C4
Chloris sp.	grass	C4
Cortaderia selloana (Schult. & Schult. f.) Asch. & Graebn.	grass	C3
Cynodon dactylon (L.) Pers. var. dactylon	grass	C4
Deyeuxia hieronymi (Hack.) Türpe	grass	C3
Digitaria californica (Benth.) Henrard var. californica	grass	C4
Digitaria sacchariflora (Nees) Henrard	grass	C4
Digitaria sanguinalis (L.) Scop.	grass	C4
Distichlis scoparia (Kunth) Arechav. var. scoparia	grass	C4
Distichlis spicata (L.) Greene var. spicata	grass	C4
Eleusine tristachya (Lam.) Lam.	grass	C4
Eragrostis curvula (Schrad.) Nees	grass	C4
Eragrostis lugens Nees	grass	C4
Eragrostis mexicana (Hornem.) Link ssp. mexicana	grass	C4

Table 1 (continued)

Family/species	Life form	Metabolic pathway
Eustachys retusa (Lag.) Kunth	grass	C4
Festuca hieronymi Hack. var. hieronymi	grass	C3
Gouinia paraguayensis (Kuntze) Parodi	grass	C4
Hordeum stenostachys Godr.	grass	C3
Jarava plumosa (Spreng.) S. W. L. Jacobs & J. Everett	grass	C3
Jarava pseudoichu (Caro) F. Rojas	grass	C3
Leptochloa chloridiformis (Hack.) Parodi	grass	C4
Lolium perenne L.	grass	C3
Muhlenbergia peruviana (P. Beauv.) Steud.	grass	C4
Nassella hyalina (Nees) Barkworth	grass	C3
Nassella tenuissima (Trin.) Barkworth	grass	C3
Nassella trichotoma (Nees) Hack. ex Arechav.	grass	C3
Neobouteloua lophostachya (Griseb.) Gould	grass	C4
Pappophorum pappiferum (Lam.) Kuntze	grass	C4
Paspalum dilatatum (Poir.) ssp. dilatatum	grass	C4
Paspalum malacophyllum Trin.	grass	C4
Paspalum notatum Flüggé	grass	C4
Paspalum quadrifarium Lam.	grass	C4
Piptochaetium sp.	grass	C3
Poa sp	grass	C3
Setaria lachnea (Nees) Kunth	grass	C4
Setaria oblongata (Griseb.) Parodi	grass	C4
Setaria pampeana Parodi ex Nicora	grass	C4
Setaria parviflora (Poir.) Kerguélen	grass	C4
Sorghum halepense (L.) Pers.	grass	C3
Spartina spartinae (Trin.) Merr. ex Hitchc.	grass	C3
Sporobolus indicus (L.) R. Br.	grass	C4
Sporobolus pyramidatus (Lam.) Hitchc.	grass	C4
Tragus berteroniamus Schult.	grass	C4
Trichloris crinita (Lag.) Parodi	grass	C4
Polygonaceae		
Rumex acetosa L.	herb	C3
Portulacaceae		
Portulaca oleraceae L.	herb	C3
Talinum fruticosum (L.) Juss.	herb	C3
Talinum paniculatum (Jacq.) Gaertn.	herb	C3
Talinum polygaloides Gillies ex Arn.	herb	C3
Ranunculaceae		
Clematis montevidense Spreng.	epiphyte	C3
Rhamnaceae		
Condalia buxifolia Reissek	shrub	C3
Condalia microphylla Cav.	shrub	C3
Condalia montana A. Cast.	shrub	C3
Ziziphus mistol Griseb.	tree	C3

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Table 1 (continued)

Family/species	Life form	Metabolic pathway
Rosaceae		
Lachemilla pignata (Ruiz & Pav.) Rothm.	herb	C3
Rubiaceae		
Borreria eryngioides var. ostenii (Standl.) E.L. Cabral & Bacigalupo	herb	C3
Galium richardianum (Gillies ex Hook. & Arn.) Endl. ex Walp.	herb	C3
Mitracarpus megapotamicus (Spreng.) Kuntze	herb	C3
Rutaceae		
Zanthoxylum coco Gillies ex Hook. f. et Arn.	tree	C3
Santalaceae		
Jodina rhombifolia (Hook. & Arn.) Reissek	shrub	C3
Sapindaceae		
Cardiospermum halicacabum L.	epiphyte	C3
Urvillea chacoënsis Hunz.	epiphyte	C3
Selaginellaceae		
Selaginella peruviana (Milde) Hieron.	fern	C3
Selaginella sellowii Hieron.	fern	C3
Simaroubaceae		
Castela coccinea Griseb.	tree	C3
Solanaceae		
Capsicum chacoënse Hunz.	herb	C3
Cestrum parqui L'Hér.	shrub	C3
Datura ferox L.	herb	C3
Grabowskia aff. duplicata Arn.	shrub	C3
Lycium chilense Miers ex Bertero	shrub	C3
Lycium ciliatum Schltdl.	shrub	C3
Lycium elongatum Miers.	shrub	C3
Lycium gilliesianum Miers	shrub	C3
Nierembergia aristata D. Don	herb	C3
Petunia axillaris (Lam.) Britton, Stern & Poggenb.	herb	C3
Salpichroa origanifolia (Lam.) Baill.	herb	C3
Solanum angustifidum Bitter	herb	C3
Solanum argentinum Bitter & Lillo	herb	C3
Solanum chenopodioides Lam.	herb	C3
Solanum elaeagnifolium Cav.	herb	C3
Solanum sisymbriifolium Lam.	herb	C3
Solanum stuckertii Bitter	herb	C3
Vassobia breviflora (Sendtn.) Hunz.	shrub	C3
Urticaceae		
Parietaria debilis G. Forst.	herb	C3
Urtica sp.	herb	C3
Verbenaceae		
Aloysia gratissima (Gillies & Hook. ex Hook.) Tronc.	shrub	C3
Glandularia peruviana (L.) Small	herb	C3
Lantana grisebachii Seckt var. grisebachii	herb	C3

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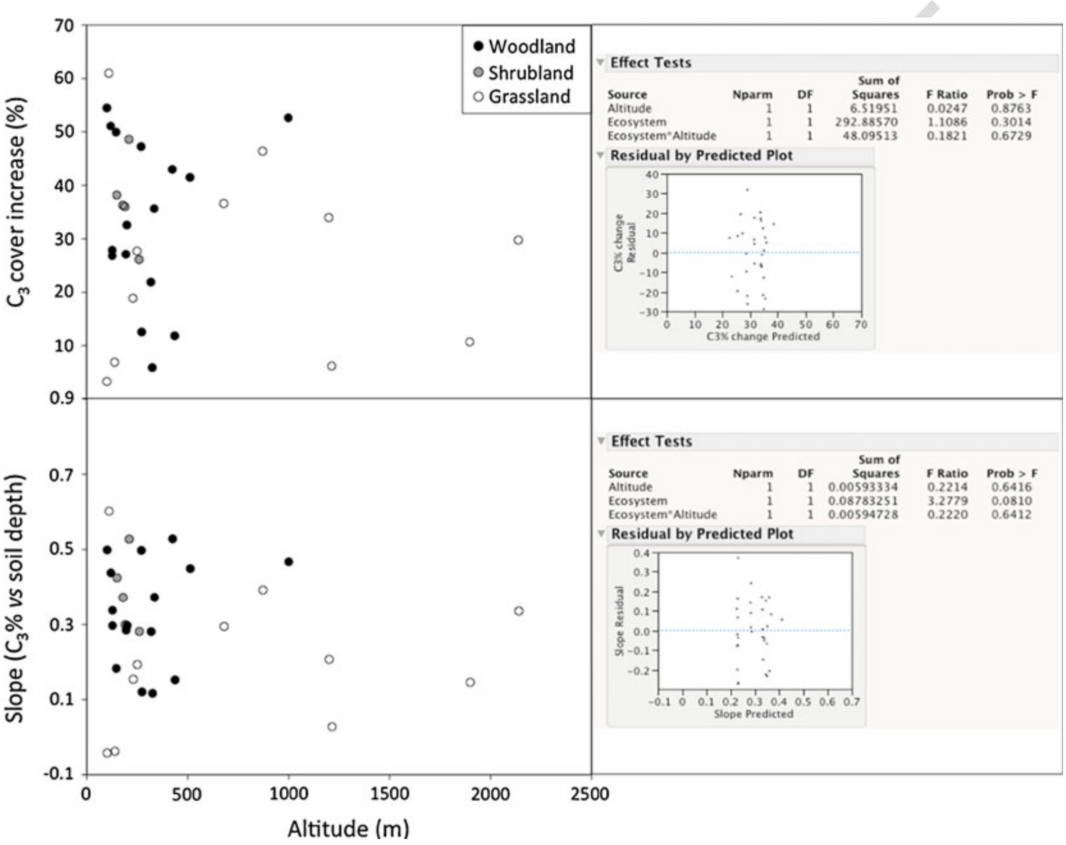
Table 1 (continued)

Family/species	Life form	Metabolic pathway
Lippia turbinata Griseb. f. turbinata	shrub	C3
Phyla canescens (Kunth) Greene	herb	C3
Verbena bonariensis L.	herb	C3
Zygophyllaceae		
Larrea divaricata Cav.	shrub	C3
Porlieria mycrophylla (Baill.) Descole, O'Donell & Lourteig	shrub	C3

Appendix 2



Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Altitude	1	1	6.51951	0.0247	0.8763
Ecosystem	1	1	292.88570	1.1086	0.3014
Ecosystem*Altitude	1	1	48.09513	0.1821	0.6729

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Altitude	1	1	0.00593334	0.2214	0.6416
Ecosystem	1	1	0.08783251	3.2779	0.0810
Ecosystem*Altitude	1	1	0.00594728	0.2220	0.6412

Q5

Q6

Fig. 6 Changes in vegetation cover through time across ecosystems and altitudes, as measured by percent differences between the C_3/C_4 relative cover estimated in superficial (0–1 cm) and in deep soils (50 cm) and by slope (angular coefficient) of least square regressions, performed between estimates of C_3/C_4 cover and their respective soil depth at each study site. Note that no significant effects on vegetation change are observed for ecosystem, altitude or their interaction

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