Martínez-Vilalta, Sala, Asensio, Galiano, Hoch, Palacio, Piper and Lloret. Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis. *Ecological Monographs*.

APPENDIX S3. Detailed comparison of different methods for NSC extraction and assessment of their possible influence on reported results.

APPROACH

Whenever possible, for each study included in our analyses we categorized the extraction and quantification methods for starch and soluble sugars (SS), largely following the classification used by Quentin et al. (2015) (Table C1). When necessary, original references were checked to assess methods. The following categories were used; for starch extraction: Acid (HCl, $HClO_4$ or H_2SO_4) or Enzymatic (amyloglucosidase or amyloglucosidase + α-amylase); for SS extraction: Acetone, Alcohol (ethanol or methanol), Mixes (basically methanol : chloroform : water) or Water; for starch or SS quantification: Chromatographic, Colorimetric (when absorbance was measured in the visible light spectrum) or Enzymatic (when glucose-6-phosphate dehydrogenase and glucose hexokinase were used). We also used a more detailed classification for the starch and SS quantification (level 2) based on the specific method used (Chromatographic, Anthrone, Phenol-sulphuric, Glucose hexokinase, Glucose peroxidase or Other). However, this information was not provided by all studies and, therefore, overall sample size was reduced when this more detailed classification was used. Table C1 provides the minimum number of studies using each extraction and quantification method.

In a first set of analyses, we tested if including the extraction and quantification methods improved the mixed effects linear models (GLMM) used to analyze the effect of organ, organ x functional type and organ x biome on the mean values of our three main response variables: soluble sugars (SS) concentration, starch concentration, and their sum (NSC_T). The structure of these models was identical to

the one described in the *Data analyses* section of the main text, except that the variables characterizing extraction and quantification methods of SS and starch were included as fixed factors, one at a time. We did not include multiple methodological variables in the same model because these variables were clearly not independent from each other. Overall, we fitted 27 models, resulting from combinations of response variable (SS, starch or NSC_T), model structure (organ, biome or functional type) and methodological variable included (SS extraction, SS level 1 quantification, starch extraction or starch level 1 quantification) (Table C2). Based on results from Quentin et al. (2015), we did not test for starch extraction or quantification effects on SS concentrations, or for SS quantification effects on starch concentrations. Whenever including a certain methodological variable improved significantly the fit of the basic model (according to a likelihood ratio test), we assessed to what degree the significant effects in the new model differed from those corresponding to the base model as reported in the main text (without methodological information). Results were qualitatively identical when the more detailed (level 2) classification was used instead of the simpler (level 1) classification of quantification methods, and are not reported here.

RESULTS

Out of the 27 combinations we tested, including a methodological variable improved the fit of the base model in only four cases (Table 1, main text). Starch was the only response variable affected, and only by extraction methods (SS extraction in one case and starch extraction in three cases; Figures C1 and C2). The effect of starch extraction was always in the same direction, with acid extractions resulting in higher starch concentrations than enzymatic methods ($P < 0.05$ in all three cases; Figure C2). Although including SS extraction improved the biome model for starch, individual SS extraction methods did not significantly affect starch estimates ($P > 0.05$ for all pairwise comparisons). Only water extractions of SS produced higher starch estimates, but differences were only marginally significant ($P = 0.082$; Figure C1).

Including (level 1) quantification methods did not result in significant improvements in model fit (Table 1, main text), indicating that there were no consistent differences among quantification approaches.

In each of the four cases in which including methodological effects improved model fit (Table 1, main text), we assessed whether the model including methodological effects differed in any meaningful way from the base model reported in the main text. In three cases model results were identical, and only in one case (the starch model including organ, functional type and starch extraction as fixed factors) we found a minor difference compared to what is reported in the main text. In this case, when the effect of starch extraction was included, starch concentrations in the stems of herbaceous species were no longer significantly different from those of conifer stems; that is, the letter code corresponding to herbaceous species in the central panel of Figure 3 (main text) would be 'AB' instead of 'B'. This difference has no impact in our conclusions. Overall, the fact that results remained nearly identical when methodological variables were accounted for, gives us confidence that our conclusions pertaining to average values are not confounded by methodological effects.

TABLE S1**.** Number of studies by method used to extract and quantify non-structural carbohydrates.

Variable	**Level**	**N**
Starch extraction (18 NAs)	Acid	41
	Enzymatic	65
Soluble sugars extraction (26 NAs)	Acetone	6
	Alcohol	48
	Mixes	15
	Water	29
Starch quantification – Level 1 (12 NAs)	Chromatographic	7
	Colorimetric	68
	Enzymatic	35
Soluble sugars quantification – Level 1 (16 NAs)	Chromatographic	18
	Colorimetric	61
	Enzymatic	29
Starch quantification – Level 2 (24 NAs)	Anthrone	18
	Chromatographic	7
	GHK	30
	Glucose Perox.	13
	Phenol-Sulphuric	16
	Other	16
Soluble sugars quantification – Level 2 (24 NAs)	Anthrone	20
	Chromatographic	18
	GHK	27
	Glucose Perox.	3

	Phenol-Sulphuric	24
	Other	8

NA refers to studies where there was insufficient information to classify the methods and no original references were cited, or studies where not all fractions were measured (e.g., if only starch was measured, the SS method becomes NA).

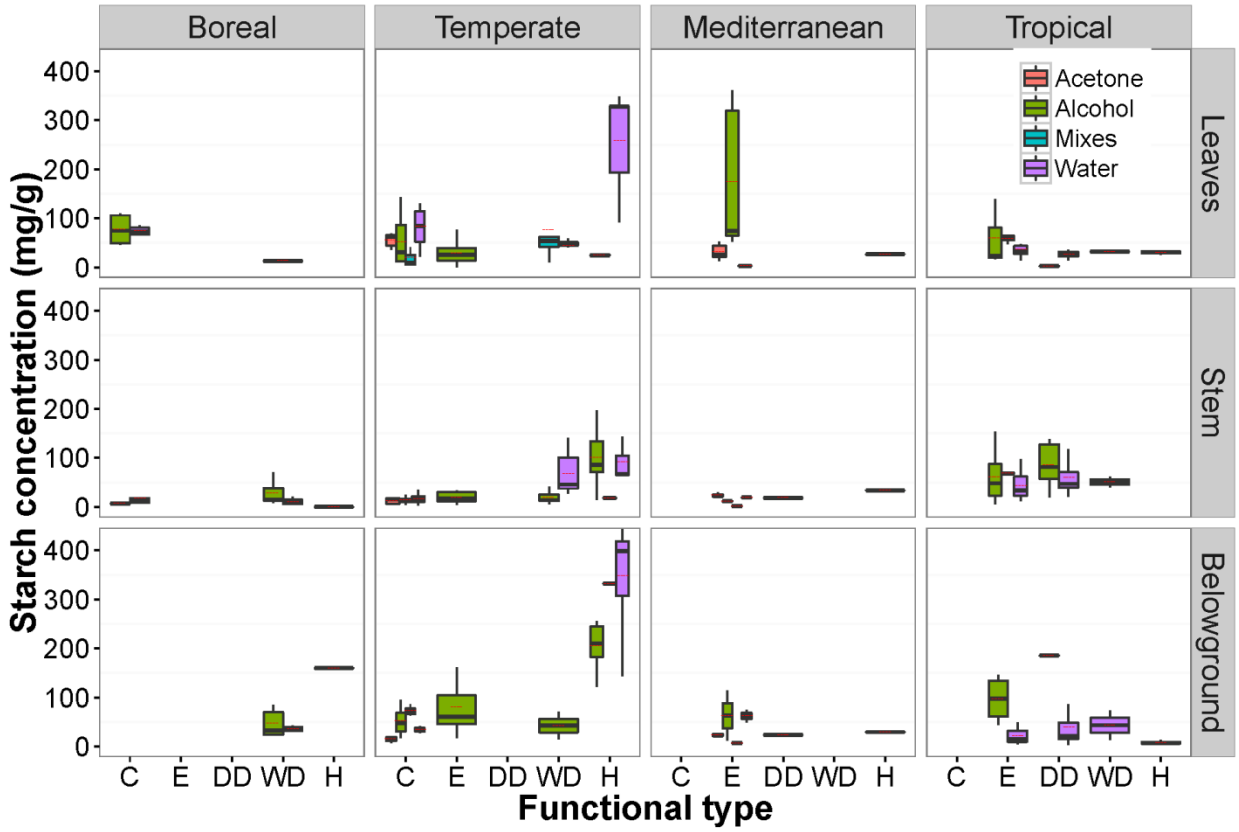

FIG. S1. Box and whiskers plot of starch concentrations as a function of biome, functional type, organ and soluble sugars' extraction method (Acetone, Alcohol, Mixes or Water). Thick horizontal bars (black) show the median, whereas fine red dashed lines indicate the mean. The upper and lower "hinges" correspond to the first and third quartiles (the 25th and 75th percentiles), and whiskers extend from the hinge to the highest (or lowest) value that is within 1.5 * IQR of the hinge. All these statistics are computed across species by context combinations (context corresponds to different combinations of study, site and specific measurement conditions; see text).

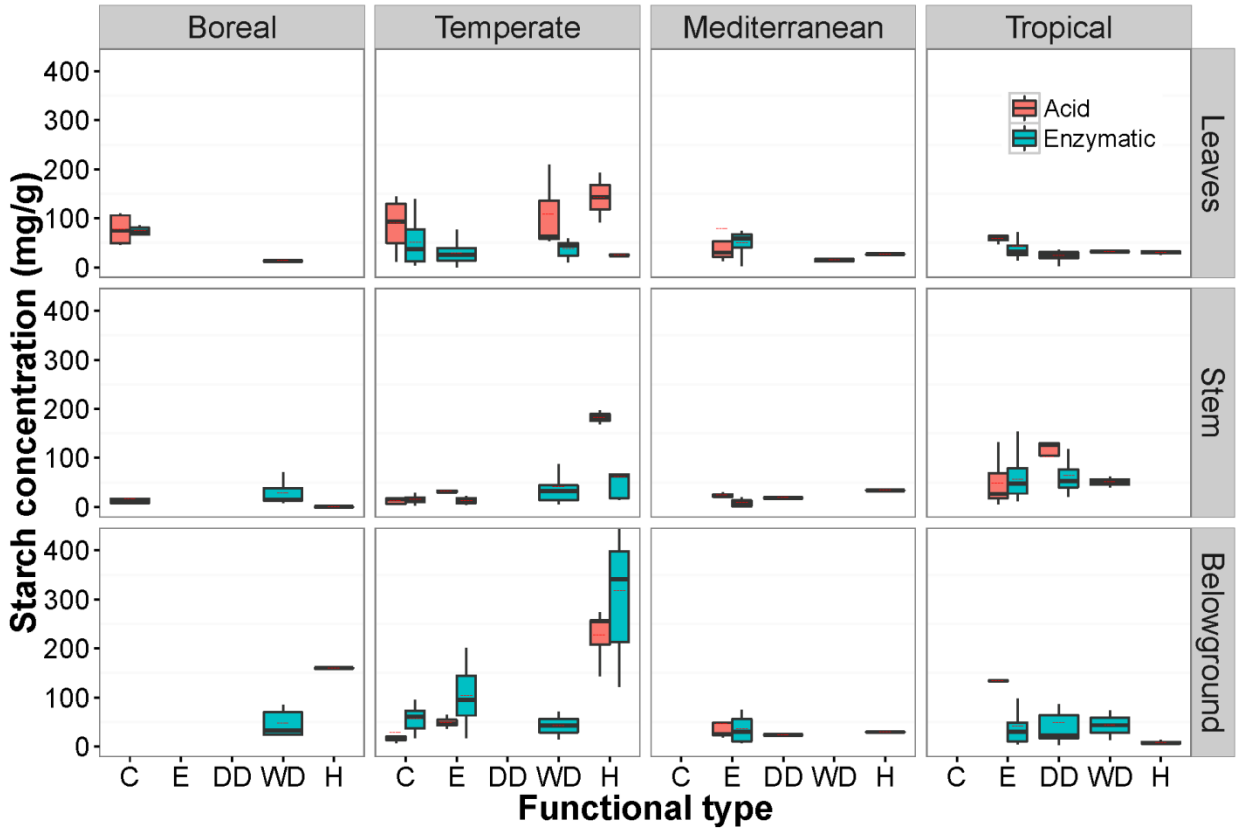

FIG. S2**.** Box and whiskers plot of starch concentrations as a function of biome, functional type, organ and starch extraction method (Acid or Enzymatic). Thick horizontal bars (black) show the median, whereas fine red dashed lines indicate the mean. The upper and lower "hinges" correspond to the first and third quartiles (the 25th and 75th percentiles), and whiskers extend from the hinge to the highest (or lowest) value that is within 1.5 * IQR of the hinge. All these statistics are computed across species by context combinations (context corresponds to different combinations of study, site and specific measurement conditions; see text).